

N° d'enregistrement  
au C.N.R.S.

AO 12313

Article principal  
recouvrant en partie

la T H E S E présentée  
pour l'obtention

du grade de DOCTEUR ES SCIENCES  
NATURELLES

A L'UNIVERSITE Pierre et Marie CURIE

(PARIS VI)

par Monsieur DUJON Bernard

Sujet de la thèse : Recombinaison, ségrégation et mutation  
des gènes mitochondriaux chez la levure *Saccharomyces  
cerevisiae*.

soutenue le : 5 Mars 1976

devant la Commission d'examen composée de :

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# Mitochondrial Genetics X: Effects of UV Irradiation on Transmission and Recombination of Mitochondrial Genes in *Saccharomyces cerevisiae*

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Received November 12, 1974

**Summary.** UV irradiation has been applied either to one parent prior to crossing or to newly formed zygotes. The effects of UV have been studied on a) the transmission of mitochondrial alleles at the loci conferring resistance to chloramphenicol, erythromycin, oligomycin or paromycin, b) the frequency of recombinants between various combinations of alleles at these loci. The effects of UV depend on the nature of the cross i.e. homosexual ( $\omega^+ \times \omega^+$  or  $\omega^- \times \omega^-$ ) or heterosexual ( $\omega^+ \times \omega^-$ ). In all cases UV irradiation of one of the parents diminishes the transmission (output) of the mitochondrial alleles originated from the irradiated parent. In homosexual crosses the decrease of transmission is the same for alleles at all the loci: there is a coordinated output. In heterosexual crosses, when the  $\omega^+$  parent is irradiated, there is a differential decrease of transmission depending on the distance of the resistance locus relative to the  $\omega$  locus. In heterosexual crosses irradiation of the  $\omega^+$  parent increases the frequency of recombinants while irradiation of the  $\omega^-$  parent slightly decreases it. In homosexual crosses the frequency of recombinants diminishes when a high UV dose is applied to one of the parents. No or only minor modifications of the polarity of recombination are observed. Irradiation of newly formed zygotes has no or minor effects on the transmission of alleles and recombinant frequencies.

All these effects can be interpreted in terms of a general model for recombination of mitochondrial genes. This model stresses that the mitochondrial genetics is the genetics of a population of mit-DNA molecules forming a panmictic pool characterized by a variable input fraction and undergoing several random in time mating rounds. Genetic recombination between mit-DNA molecules occurs all the time and results from non reciprocal elementary acts of molecular recombination.

UV irradiation of one of the parents leads to a modification of the input fraction in favor of the non irradiated parent. As a consequence of this modification the output of alleles and the frequency of recombinants are changed. A good quantitative agreement between the predictions calculated on the basis of the model and the experimental data is found. Relationships between the molecular events responsible for the modifications of input and the production of  $q^-$  primary clones by UV are discussed.

## Introduction

Numerous results concerning the segregation and recombination of mitochondrial genes in *Saccharomyces cerevisiae* have appeared in the recent literature (Avner *et al.*, 1973; Bolotin *et al.*, 1971; Callan, 1974; Coen *et al.*, 1970; Howell *et al.*, 1973; Kleese *et al.*, 1972a and b; Linnane *et al.*, 1968; Lukins *et al.*, 1973;

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Michaelis *et al.*, 1973; Mitchell *et al.*, 1972; Netter *et al.*, 1974; Rank, 1973; Rank and Bech Hansen, 1972; Saunders *et al.*, 1971; Shannon *et al.*, 1972; Suda and Uchida, 1972; Suda and Uchida, 1974; Thomas and Wilkie, 1968; Trembath *et al.*, 1973; Wakabayashi and Kamei, 1973; Waxman *et al.*, 1973; Wilkie and Thomas, 1973; Wolf *et al.*, 1973). It is established now that: a) segregation is relatively rapid so that pure homozygous cell lines appear within a few cell generations from an initially heterozygous cell, b) genetic recombination occurs frequently, c) both genetically linked and unlinked loci have been described on the same mit-DNA<sup>1</sup> molecule, d) there exist two types of crosses determined by the two alleles of the mitochondrial locus  $\omega$ , which governs the polarity of recombination between markers located in the polar region, e) the mitochondrial genetics is the genetics of a population of molecules. Most of these results have been obtained from analyses of the progenies of crosses which have not been modified by the action of an effector. It seemed obvious to us that one can get more insight into the mechanisms of segregation and recombination of mitochondrial genes by studying the influence of effectors on these processes.

Since the experiments of Haas *et al.* (1948) UV irradiation has become a classical effector of genetic recombination as well in eucaryotes as in phages and bacteria. UV irradiation has also been applied to study of non mendelian genetics in *Chlamydomonas* (Sager and Ramanis, 1967; Gillham *et al.*, 1974). Furthermore, UV irradiation has been shown to be an effective inducer of respiratory deficient mutations ( $q^-$ ) in yeast (Raut and Simpson, 1955; Maroudas and Wilkie, 1968; Allen and McQuillan, 1969; Moustacchi, 1969, 1972) suggesting that UV affects the mit-DNA molecules. These facts prompted us to examine the effects of UV on transmission and recombination of mitochondrial genes.

UV effects can be studied by means of two experimental designs: 1) UV is applied to a population of cells heterozygous for mitochondrial genes and undergoing segregation and recombination processes. Experimentally, this consists in an irradiation of cell zygotes just *after* their formation. 2) UV is applied to a population of cells homozygous for mitochondrial genes followed by cell fusion leading to mitochondrial heterozygosity and to processes of segregation and recombination. Experimentally this consists in an irradiation of haploid parent *prior* to the formation of cell zygotes. In both cases the progeny of mitochondrial crosses is analysed many cell generations later, after genetic recombination and segregation events have already taken place. Both approaches have been used in this work. As it will be shown, the first one does not modify significantly the results of crosses while the second one drastically changes the frequencies of different genetic classes amongst the diploid progeny of the cross. Therefore most of the work will be devoted to the second approach. The aim of this paper is twofold: 1) a systematic description of the UV effects applied to one or the other parent prior to mating, 2) an attempt to interpret the effects of UV in terms of the model for segregation and recombination of mitochondrial genes recently proposed by Dujon *et al.* (1974).

Results presented here show that UV irradiation of one parent prior to mating has two major effects: it diminishes the transmission of mitochondrial alleles

1 mit-DNA: mitochondrial deoxyribonucleic acid.

carried by the irradiated parent; it either increases or decreases the frequency of recombinants depending on the nature of the cross  $\omega^+ \times \omega^+$ ,  $\omega^- \times \omega^-$ ,  $\omega^+ \times \omega^-$  and the nature of allele present at the  $\omega$  locus in the irradiated parent. The first effect is interpreted as a diminution of the input of mit-DNA molecules of the irradiated parent which enter the mating pool, while the second effect is, as predicted by the model, a consequence of this diminution. UV irradiation prior to mating acts as an effector which modifies the proportions of various types of mit-DNA molecules which undergo genetic exchanges in the pool. It has no, or only minor effects on the frequency of genetic exchanges in already paired molecules.

## Material and Methods

### *Strains*

The strains used are described in Table 1.

### *Media*

The media designated W0, N0, N2, N3, N3C, N3E and N3CE have been described by Coen *et al.* (1970), and Deutsch *et al.* (1974); N3O and N3CO by Avner *et al.* (1973); K3P and K3OP by Wolf *et al.* (1973).

### *Irradiation Procedures*

Haploid strains were grown at 28° C in liquid selective medium (N3C, N3E, N3CE, N3O or K3P according to their genotype) for 48 hours in shaken cultures. Stationary phase cultures were harvested, washed at least once in saline and resuspended in cold saline at a concentration of  $10^7$  cells/ml.

A Petri dish containing 10 ml of an ice cold suspension was submitted to irradiation by a General Electric model G15 T8 Germicidal lamp (15 watts). To ensure a homogeneous dose distribution the liquid within the Petri dish was stirred throughout the irradiation process.

All irradiations were carried out at intensity of  $16 \text{ ergs mm}^{-2} \text{ sec}^{-1}$  as measured by a Latarjet dosimeter, exposure time varied in most experiments from 0 to 100 seconds. Immediately after irradiation the cell suspensions were poured back into a tube and kept in the darkness at ice temperature throughout the dilution and plating procedures.

### *Survival of Irradiated Haploids and Frequency of Petites Induced*

Irradiated suspensions were diluted in saline solution, plated on N2 medium (for grandes versus petites discrimination) and incubated 6 to 7 days at 28° C in darkness. In some cases replica platings on antibiotic containing media and replica crosses [cf. Deutsch *et al.* (1974)] have been performed using N2 plates as master plates (see Fig. 1).

### *Quantitative Random Analysis of Crosses with the Irradiated Haploid Cells*

Crosses were performed by the lawn technique. Minimal medium plates were spread with a lawn of  $5 \cdot 10^6$  to  $5 \cdot 10^7$  cells of the non irradiated parent; this constituted the lawn on which the suspension of the irradiated parent was plated (0.1 or 0.2 ml of the irradiated suspension itself or of a dilution of it). The plates were incubated three days in the darkness at 28° C. According to the strain, the UV dose and the individual cross performed,  $10^2$  to  $10^4$  prototrophic colonies issued from individual zygotes were obtained per Petri dish. These zygotic colonies are composed of diploid cells which have undergone some 20 generations since the copulation. The colonies were harvested by spreading the plate with a few milliliters of saline solution. The resulting suspension which represents a randomized population of diploid cells was diluted and plated on non selective glucose minimal medium (W0). The minimal medium plates were incubated for three days at 28° C, and then replica-plated onto various selective media, which in turn were incubated 4 to 5 days at 28° C (see Fig. 1).

### *Irradiation of Zygotes*

Synchronous zygotes were prepared by a technique derived from that of Jakob (1962). Cellular suspensions in N0 medium containing about  $10^6$  to  $10^7$  cells of each parent per milli-

Table 1. List of strains

| Strain      | Chromosomal genotype         | Mitochondrial genotype                                                                                            | Origin of the strain                    |
|-------------|------------------------------|-------------------------------------------------------------------------------------------------------------------|-----------------------------------------|
| 55R5-3C/221 | <i>a ura1</i>                | $\omega^-$ C <sup>S</sup> E <sup>R</sup> <sub>221</sub> O <sup>S</sup> P <sup>S</sup>                             | Mitoch. mutant of 55R5-3C <sup>a</sup>  |
| 55R5-3C/321 | <i>a ura1</i>                | $\omega^-$ C <sup>R</sup> <sub>321</sub> E <sup>S</sup> O <sup>S</sup> P <sup>S</sup>                             | Mitoch. mutant of 55R5-3C <sup>a</sup>  |
| DP1-1B/7    | $\alpha$ <i>his1 trp1</i>    | $\omega^+$ C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> <sub>7</sub> P <sup>S</sup>                               | Mitoch. mutant of DP1-1B <sup>b</sup>   |
| DP1-1B/514  | $\alpha$ <i>his1 trp1</i>    | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>514</sub> O <sup>S</sup> P <sup>S</sup>                             | Mitoch. mutant of DP1-1B <sup>a</sup>   |
| DP1-1B/553  | $\alpha$ <i>his1 trp1</i>    | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>553</sub> O <sup>S</sup> P <sup>S</sup>                             | Mitoch. mutant of DP1-1B <sup>aa</sup>  |
| D22/A15     | <i>a ade2</i>                | $\omega^+$ C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> <sub>144</sub> P <sup>S</sup>                             | Mitoch. mutant of D22 <sup>b</sup>      |
| D22/A16     | <i>a ade2</i>                | $\omega^+$ C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> <sub>145</sub> P <sup>S</sup>                             | Mitoch. mutant of D22 <sup>b</sup>      |
| M12/54      | <i>a ilv5 trp2 ura3 aux-</i> | $\omega^-$ C <sup>S</sup> E <sup>S</sup> O <sup>S</sup> P <sup>R</sup> <sub>454</sub>                             | Mitoch. mutant of M12 <sup>c</sup>      |
| LM650-2C/36 | $\alpha$ <i>his1 trp1</i>    | $\omega^+$ C <sup>R</sup> <sub>36</sub> E <sup>S</sup> O <sup>S</sup> P <sup>S</sup>                              | Mitoch. mutant of LM650-2C <sup>a</sup> |
| IL8-8D      | <i>a ura1</i>                | $\omega^+$ C <sup>R</sup> <sub>321</sub> E <sup>R</sup> <sub>514</sub> O <sup>S</sup> P <sup>S</sup>              | Meiosis of IL8 <sup>bb</sup>            |
| IL16-10B    | $\alpha$ <i>his1</i>         | $\omega^-$ C <sup>R</sup> <sub>321</sub> E <sup>S</sup> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL16 <sup>bb</sup>           |
| IL16-11D    | <i>a ura1</i>                | $\omega^-$ C <sup>R</sup> <sub>321</sub> E <sup>S</sup> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL16 <sup>bb</sup>           |
| IL17-1D     | $\alpha$ <i>his1 trp1</i>    | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>514</sub> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL17 <sup>bb</sup>           |
| IL17-4A     | <i>a ura1</i>                | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>514</sub> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL17 <sup>bb</sup>           |
| IL17-9B     | <i>a ura1</i>                | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>514</sub> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL17 <sup>bb</sup>           |
| IL102-8D    | $\alpha$ <i>his1 trp1</i>    | $\omega^-$ C <sup>S</sup> E <sup>R</sup> <sub>221</sub> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL102 <sup>bb</sup>          |
| IL126-1C    | <i>a ura1</i>                | $\omega^-$ C <sup>R</sup> <sub>321</sub> E <sup>R</sup> <sub>221</sub> O <sup>S</sup> P <sup>S</sup>              | Meiosis of IL126 <sup>bb</sup>          |
| IL126-3A    | $\alpha$ <i>his1</i>         | $\omega^-$ C <sup>R</sup> <sub>321</sub> E <sup>R</sup> <sub>221</sub> O <sup>S</sup> P <sup>S</sup>              | Meiosis of IL126 <sup>bb</sup>          |
| IL187-7A    | $\alpha$ <i>his1</i>         | $\omega^+$ C <sup>R</sup> <sub>321</sub> E <sup>S</sup> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL187 <sup>bb</sup>          |
| IL216-1B    | <i>a ura1 trp1</i>           | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>221</sub> O <sup>S</sup> P <sup>S</sup>                             | Meiosis of IL216 <sup>bb</sup>          |
| IL779-1C    | <i>a ura1</i>                | $\omega^+$ C <sup>S</sup> E <sup>R</sup> <sub>221</sub> O <sup>R</sup> <sub>1</sub> P <sup>S</sup>                | Meiosis of IL779 <sup>b</sup>           |
| IL836-2A    | <i>a ura1</i>                | $\omega^+$ C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> <sub>1</sub> P <sup>S</sup>                               | Meiosis of IL836 <sup>b</sup>           |
| KL14-4A     | <i>a his1 trp2</i>           | $\omega^+$ C <sup>R</sup> <sub>321</sub> E <sup>S</sup> O <sup>R</sup> <sub>1</sub> P <sup>R</sup> <sub>454</sub> | Meiosis of KL14 <sup>c</sup>            |
| PS189-3A    | $\alpha$ <i>arg</i>          | $\omega^-$ C <sup>R</sup> <sub>321</sub> E <sup>R</sup> <sub>221</sub> O <sup>S</sup> P <sup>S</sup>              | Meiosis of PS189 <sup>b</sup>           |

<sup>a</sup> Coen *et al.*, 1970.<sup>b</sup> Avner *et al.*, 1973.<sup>c</sup> Wolf *et al.*, 1973.<sup>aa</sup> Netter *et al.*, 1974.<sup>bb</sup> Coen *et al.* (in prep.).

liter were incubated at 28° C for 1 h 15' to 2 h with shaking in large flasks to ensure adequate aeration. Cells were pelleted down by centrifugation and kept for 30 to 40 minutes at room temperature without shaking. Then the pellets were resuspended into N0 medium and incubated at 28° C with shaking. Samples were withdrawn at various time intervals, diluted and plated onto W0 medium to determine the number of zygotes formed. Samples used for irradiation were washed and resuspended in cold saline at a density of about  $2 \times 10^7$  cells/ml.

Irradiations were performed with the same procedure as for haploids.

### *Quantitative Random Analysis of Crosses in which Zygotes were Irradiated*

Irradiated suspensions were diluted, plated onto W0 medium and incubated 3 days at 28° C in the darkness. Colonies issued from irradiated zygotes were scored to determine % of survival of irradiated zygotes and were harvested by spreading the plate with a few milliliters of saline solution. The resulting suspension which represents a randomized population of diploid cells was diluted and plated on non selective glucose minimal medium (W0). The W0 plates were incubated 3 days at 28° C and then replica plated onto various antibiotic media, which in turn were incubated 4 to 5 days at 28° C.

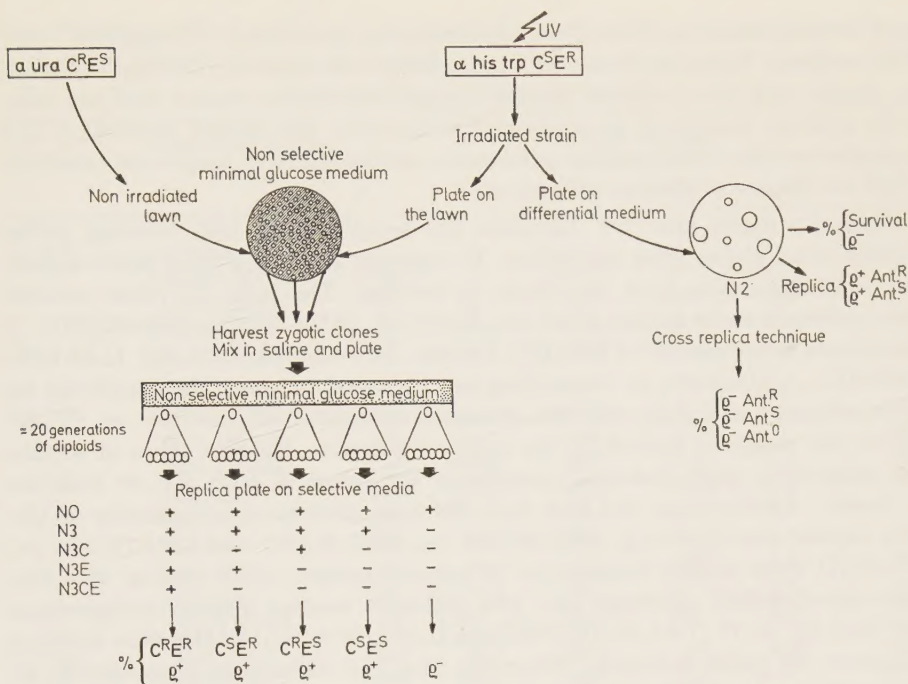


Fig. 1. Design of the experiments. See text (Material and Methods)

### Abbreviations

- $C^S/C^R$  allelic forms of the  $R_I$  locus conferring chloramphenicol sensitivity/resistance.  
 $E^S/E^R$  allelic forms of either  $R_{II}$  or  $R_{III}$  loci conferring erythromycin sensitivity/resistance.  
 $O^S/O^R$  allelic forms of either  $O_I$  or  $O_{II}$  loci conferring oligomycin sensitivity/resistance.  
 $P^S/P^R$  allelic forms of the  $P_I$  locus conferring paromomycin sensitivity/resistance.  
 $\omega^+/\omega^-$  allelic forms of the mitochondrial sex factor.  
 $g^+/g^-$  grande/cytoplasmic petite.  
 $C^O, E^O, O^O, P^O$  deletions in  $g^-$  of the loci conferring respectively the resistance to chloramphenicol, erythromycin, oligomycin and paromomycin.

In the present work:

- $C_{36}^R, C_{321}^R$  mutations are representative of the  $R_I$  locus  
 $E_{653}^R$  mutation is representative of the  $R_{II}$  locus  
 $E_{221}^R, E_{514}^R$  mutations are representative of the  $R_{III}$  locus  
 $O_1^R, O_7^R, O_{145}^R$  mutations are representative of the  $O_I$  locus  
 $O_{144}^R$  mutation is representative of the  $O_{II}$  locus  
 $P_{454}^R$  mutation is representative of the  $P_I$  locus

In the tables presented, the actual phenotypes scored (e.g.  $C^R$  or  $C^S$ ,  $E^R$  or  $E^S$ ,  $O^R$  or  $O^S$ ,  $P^R$  or  $P^S$ ) are indicated. In the text, where appropriate in discussion of the relationship between the loci, the symbols  $R_I$ ,  $R_{II}$ ,  $R_{III}$ ,  $O_I$ ,  $O_{II}$  and  $P_I$  have been used rather than the actual phenotype they determine.

## Results

### I. Survival and Petite Induction in UV Irradiated Strains

Table 2a presents the survival of the strains used. Although some variations in UV sensitivity are observed in the set of strains used, these variations are quite

small in comparison to those found between the so called "UV sensitive" and "UV resistant" strains [Cox and Parry (1968); Moustacchi (1969)]. Therefore the nuclear and mitochondrial genetic backgrounds in the strains used are relatively uniform in respect to survival. Furthermore, the overall survival is not dependent on the mitochondrial  $\omega$  locus i.e. similar survival ranges are observed in the two classes of strains:  $\omega^+$  and  $\omega^-$ .

Table 2b shows that UV increases the frequency of cells forming petite colonies when plated after irradiation. In contrast to the survival, petite induction varies markedly from one strain to another. The same UV dose induces more petites in some strains (IL17-4A, IL187-7A, 55R5-3C/321, 55R5-3C/221...) than it does in others (DP1-1B/7, DP1-1B/514, DP1-1B/553, IL17-1D, IL16-10B, IL16-11D...). However, no correlation can be found between the sensitivity to petite induction and either the mitochondrial mutations ( $C^R$ ,  $E^R$ ,  $O^R$ , vs.  $C^S$ ,  $E^S$  etc.) or the  $\omega$  alleles carried by the irradiated strains. Various types of strains with respect to petite induction sensitivity are found in both the  $\omega^+$  and the  $\omega^-$  classes. Furthermore, the fact that different mitochondrial mutants of the same nuclear genotype (e.g. 55R5-3C/321 vs. 55R5-3C/221 and DP1-1B/514 vs. DP1-1B/7) show similar frequencies of induced petites, while strains with the same mitochondrial genotype but with different nuclear genetic backgrounds (e.g. IL17-1D vs. IL17-4A vs. IL17-9B and IL16-10B vs. IL16-11D) show striking differences for petite induction, favors the idea that differences in sensitivity to petite induction by UV are mainly due to the nuclear genetic background rather than the mitochondrial genotype. Petite induction is a complex process involving many steps, and many genetic and physiological interactions [see for example Moustacchi (1972, 1973); Mahler (1973); Perlman and Mahler (1973)]. The results presented here indicate that nuclear genes are involved in, or interfere with, the final frequency of induced petites.

Replica cross analysis of grandes and UV-induced petites is shown on Table 2c. It should be stressed that all the grande colonies issued from irradiated cells still carry the mitochondrial alleles of the parent. In contrary, some of the petite colonies carry the mitochondrial alleles of the parent while others do not. In agreement with the more extensive data presented on the induction of various deletions of mitochondrial genetic markers in petites (Deutsch *et al.*, 1974) it can be seen in Table 2c that the closely linked genetic markers  $C_{321}^R$  and  $E_{221}^R$  are either lost or maintained together.

## *II. Effects of UV Irradiation of One Parent Prior to Mating on the Outcome of the Cross*

In all experiments presented in this chapter, UV has been applied to one of the haploid parents prior to cell fusion with an unirradiated partner. Effects of UV are studied amongst the diploid progeny of the cross. The mitotic segregation of mitochondrial genes has already taken place and has led to diploid cells homozygous for mitochondrial genes.

Polarity of recombination in mitochondrial crosses is under the control of a specific mitochondrial locus  $\omega$  (Bolotin *et al.*, 1971) which determines the existence

Tables 2a-c. Survival and petite induction as a function of UV irradiation

Table 2a: Survival (in %) of the total colonies (grandes and petites) is expressed as a function of the non-irradiated control.

| Irradiated strain                                       |             | UV dose (erg mm <sup>-2</sup> ) |        |                   |        |                  | Ref.  |
|---------------------------------------------------------|-------------|---------------------------------|--------|-------------------|--------|------------------|-------|
|                                                         |             | 0                               | 320    | 640               | 960    | 1600             |       |
| $\omega^+$ C <sup>R</sup>                               | LM650-2C/36 | —                               | —      | —                 | —      | —                | Ir. 1 |
|                                                         | LM650-2C/36 | (100)                           | —      | (34.5)            | —      | (2.4)            | 2     |
|                                                         | LM650-2C/36 | 100                             | —      | 57.2              | 23.3   | 4.3              | 3     |
|                                                         | IL187-7A    | 100                             | 59.3   | 33.9              | 18.3   | 4.0              | 4     |
|                                                         | IL187-7A    | 100                             | 68.6   | 48.0              | 24.4   | 6.6              | 5     |
| $\omega^+$ E <sup>R</sup>                               | DP1-1B/514  | 100                             | 71.2   | 61.8              | 10.2   | —                | 6     |
|                                                         | DP1-1B/514  | 100                             | 71.0   | 20.0              | —      | —                | 7     |
|                                                         | DP1-1B/514  | 100                             | —      | 28.0              | 5.1    | —                | 8     |
|                                                         | DP1-1B/514  | 100                             | 77.5   | 42.7              | 12.4   | 1.3              | 9     |
|                                                         | IL17-1D     | 100                             | 61.5   | (27.3)            | (6.4)  | 0.8              | 10    |
|                                                         | IL17-4A     | 100                             | —      | 74.5              | 25.4   | 2.0              | 11    |
|                                                         | IL17-9B     | 100                             | 79.5   | 56.4              | 38.1   | 2.0              | 12    |
|                                                         | IL17-9B     | 100                             | 76.0   | 61.0              | 27.0   | 1.2              | 13    |
|                                                         | IL17-9B     | 100                             | 71.6   | 58.7              | 15.2   | 0.5              | 14    |
|                                                         | DP1-1B/553  | 100                             | 100    | 66.2              | 27.7   | 2.6              | 15    |
| $\omega^+$ O <sup>R</sup>                               | DP1-1B/7    | 100                             | 69.0   | 35.9              | 7.8    | 0.05             | 16    |
|                                                         | DP1-1B/7    | 100                             | 97.6   | 49.2              | 7.2    | —                | 17    |
|                                                         | DP1-1B/7    | 100                             | —      | 31.8              | 3.1    | 0.3              | 18    |
|                                                         | D22/A15     | 100                             | (61.6) | (27.5)            | —      | —                | 19    |
|                                                         | D22/A16     | 100                             | 76.0   | (25.3)            | (7.5)  | (0.6)            | 20    |
|                                                         | IL836-2A    | 100                             | 77.8   | 32.3              | 25.4   | 4.0              | 21    |
| $\omega^+$ C <sup>R</sup> E <sup>R</sup>                | IL8-8D      | 100                             | —      | —                 | —      | 3.4 <sup>b</sup> | 22    |
| $\omega^+$ E <sup>R</sup> O <sup>R</sup>                | IL779-1C    | 100                             | 54.3   | 29.2              | (4.4)  | 0.6              | 23    |
| $\omega^+$ C <sup>R</sup> O <sup>R</sup> P <sup>R</sup> | KL14-4A     | 100                             | —      | —                 | —      | 1.2              | 24    |
| $\omega^-$ C <sup>R</sup>                               | 55R5-3C/321 | 100                             | —      | 39.7              | 9.0    | 0.2              | 25    |
|                                                         | 55R5-3C/321 | 100                             | 84.0   | 37.0              | —      | —                | 26    |
|                                                         | 55R5-3C/321 | 100                             | —      | 41.6              | 12.4   | —                | 27    |
|                                                         | 55R5-3C/321 | (100)                           | 100    | (53.8)            | (15.6) | —                | 28    |
|                                                         | IL16-10B    | 100                             | 43.8   | 25.5              | (2.6)  | (0.2)            | 29    |
|                                                         | IL16-10B    | 100                             | 72.5   | (27.0)            | (7.2)  | 0.8              | 30    |
|                                                         | IL16-11D    | 100                             | 52.0   | 38.9              | 28.8   | 2.2              | 31    |
| $\omega^-$ E <sup>R</sup>                               | 55R5-3C/221 | (100)                           | —      | 59.0              | 18.5   | 3.2              | 32    |
|                                                         | 55R5-3C/221 | 100                             | 59.4   | 20.2              | 3.6    | 0.3              | 33    |
|                                                         | IL102-8D    | (100)                           | —      | 100               | 37.4   | 4.7              | 34    |
| $\omega^-$ P <sup>R</sup>                               | M12/54      | 100                             | —      | 51.4 <sup>a</sup> | 25.8   | 0.4              | 35    |
| $\omega^-$ C <sup>R</sup> E <sup>R</sup>                | IL126-1C    | 100                             | 62.1   | (17.1)            | 2.2    | —                | 36    |
|                                                         | PS189-3A    | 100                             | —      | 70.3 <sup>a</sup> | 24.9   | 0.2              | 37    |

<sup>a</sup> 480 ergs mm<sup>-2</sup>.

<sup>b</sup> Respectively 1.1%; 0.8%; 0.4%; for 1760, 1920 and 2080 ergs mm<sup>-2</sup>.

Tables 2a-c. Haploid strains were irradiated according to Material and Methods. Aliquots of the irradiated suspension were plated on N2 medium. After 6 to 8 days of incubation at 28°C, grandes and petites colonies were scored. Replica crosses of the colonies ( $\rho^+$  and  $\rho^-$ ) were performed in some cases.

Table 2b: Frequency of petites (in %) is referred to the total number of colonies scored (grandes and petites).

| Irradiated strain                                         |             | UV dose (erg mm <sup>-2</sup> ) |       |                  |        |                   | Ref.  |
|-----------------------------------------------------------|-------------|---------------------------------|-------|------------------|--------|-------------------|-------|
|                                                           |             | 0                               | 320   | 640              | 960    | 1600              |       |
| $\omega^+$ CR                                             | LM650-2C/36 | —                               | —     | —                | —      | —                 | Ir. 1 |
|                                                           | LM650-2C/36 | (2.1)                           | —     | (39.8)           | —      | (82.1)            | 2     |
|                                                           | LM650-2C/36 | —                               | —     | —                | —      | —                 | 3     |
|                                                           | IL187-7A    | 5.3                             | 16.3  | 51.8             | 52.9   | 62.8              | 4     |
|                                                           | IL187-7A    | 6.6                             | 26.1  | 46.5             | 56.2   | 77.5              | 5     |
| $\omega^+$ ER                                             | DP1-1B/514  | 0.0                             | 2.3   | 6.5              | 19.7   | —                 | 6     |
|                                                           | DP1-1B/514  | 0.4                             | 3.6   | 13.0             | —      | —                 | 7     |
|                                                           | DP1-1B/514  | 0.0                             | —     | 9.0              | 23.0   | —                 | 8     |
|                                                           | DP1-1B/514  | 0.8                             | 3.5   | 7.2              | 13.8   | 18.2              | 9     |
|                                                           | IL17-1D     | 0.0                             | 1.9   | (2.2)            | (10.7) | 22.9              | 10    |
|                                                           | IL17-4A     | 1.2                             | —     | 20.1             | 44.0   | 68.4              | 11    |
|                                                           | IL17-9B     | 2.2                             | 2.8   | 6.5              | 13.8   | 36.5              | 12    |
|                                                           | IL17-9B     | 1.2                             | 1.5   | 5.9              | 12.9   | 45.6              | 13    |
|                                                           | IL17-9B     | 0.4                             | 2.5   | 4.3              | 12.2   | 49.6              | 14    |
|                                                           | DP1-1B/553  | 0.0                             | 1.7   | 5.3              | 8.3    | 10.4              | 15    |
| $\omega^+$ OR                                             | DP1-1B/7    | 1.3                             | 4.8   | 5.3              | 21.5   | —                 | 16    |
|                                                           | DP1-1B/7    | 0.0                             | 3.3   | 6.0              | 20.8   | —                 | 17    |
|                                                           | DP1-1B/7    | 1.8                             | —     | 11.2             | 22.2   | 14.7              | 18    |
|                                                           | D22/A15     | 0.0                             | (1.2) | (9.3)            | —      | —                 | 19    |
|                                                           | D22/A16     | 2.9                             | 13.6  | (17.0)           | (34.6) | (48.2)            | 20    |
|                                                           | IL836-2A    | 0.0                             | 2.1   | 15.4             | 22.3   | 42.6              | 21    |
| $\omega^+$ C <sup>R</sup> E <sup>R</sup> R                | IL8-8D      | 0.3                             | —     | —                | —      | 30.3 <sup>b</sup> | 22    |
| $\omega^+$ E <sup>R</sup> O <sup>R</sup> R                | IL779-1C    | 0.0                             | 5.3   | 19.6             | (37.1) | 47.2              | 23    |
| $\omega^+$ C <sup>R</sup> O <sup>R</sup> P <sup>R</sup> R | KL14-4A     | 0.4                             | —     | —                | —      | 47.9              | 24    |
| $\omega^-$ CR                                             | 55R5-3C/321 | 4.2                             | —     | 44.4             | 69.1   | 85.8              | 25    |
|                                                           | 55R5-3C/321 | 1.5                             | 4.9   | 30.0             | —      | —                 | 26    |
|                                                           | 55R5-3C/321 | 1.0                             | —     | 35.2             | 56.5   | —                 | 27    |
|                                                           | 55R5-3C/321 | (0.0)                           | 19.0  | (31.4)           | (69.2) | —                 | 28    |
|                                                           | IL16-10B    | 0.7                             | 3.6   | 16.1             | (30.1) | (24.0)            | 29    |
|                                                           | IL16-10B    | 0.9                             | 5.9   | (18.3)           | (23.0) | 17.3              | 30    |
|                                                           | IL16-11D    | 0.0                             | 0.0   | 3.5              | 6.8    | 18.5              | 31    |
| $\omega^-$ ER                                             | 55R5-3C/221 | (2.1)                           | —     | 40.0             | 66.7   | 80.2              | 32    |
|                                                           | 55R5-3C/221 | 2.2                             | 7.8   | 23.9             | 42.0   | 68.6              | 33    |
|                                                           | IL102-8D    | (1.4)                           | 10.8  | 22.3             | 35.3   | —                 | 34    |
| $\omega^-$ PR                                             | M12/54      | 0.1                             | —     | 4.0 <sup>a</sup> | 21.5   | 52.4              | 35    |
| $\omega^-$ C <sup>R</sup> E <sup>R</sup> R                | IL126-1C    | 2.4                             | 8.2   | (7.8)            | 17.0   | —                 | 36    |
|                                                           | PS189-3A    | 0.3                             | —     | 2.5 <sup>a</sup> | 17.1   | 76.4              | 37    |

<sup>a</sup> 480 ergs mm<sup>-2</sup>.

<sup>b</sup> Respectively 23.8, 21.4 and 27.4 for 1760, 1920 and 2080 ergs mm<sup>-2</sup>.

Tables 2a—c. continued. Each experiment of irradiation (a series of UV doses on the same cell suspension) has been given a number (Ir.) for reference to Tables 3, 4, 5 and 6. In every case (except for frequencies shown in parenthesis, more than 100 colonies have been scored (365 in average). For numbers in parentheses between 50 and 100 colonies have been scored.

Table 2c. Analysis of genotypes of haploid colonies issued from irradiated cells. Number of colonies ( $\varrho^+$  or  $\varrho^-$ ) corresponding to genotype indicated.

| Irradiated strains    | Tester strain used for replica cross | Deduced genotype of                                                                |                                         |
|-----------------------|--------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------|
|                       |                                      | $\varrho^-$ colonies                                                               | $\varrho^+$ colonies                    |
| 55R5-3C/321           | DP1-1B/514                           | C <sup>R</sup> :C <sup>O</sup><br>0:54                                             | C <sup>R</sup> :C <sup>O</sup><br>123:0 |
| LM650-2C/36           | 55R5-3C/221                          | C <sup>R</sup> :C <sup>O</sup><br>8:5                                              | C <sup>R</sup> :C <sup>O</sup><br>158:0 |
| 55R5-3C/221           | LM650-2C/36                          | E <sup>R</sup> :E <sup>O</sup><br>2:91                                             | E <sup>R</sup> :E <sup>O</sup><br>136:0 |
| DP1-1B/514            | 55R5-3C/321                          | E <sup>R</sup> :E <sup>O</sup><br>46:13                                            | E <sup>R</sup> :E <sup>O</sup><br>329:0 |
|                       | 55R5-3C/323                          |                                                                                    |                                         |
| DP1-1B/7              | IL102-9D                             | O <sup>R</sup> :O <sup>O</sup><br>12:2                                             | O <sup>R</sup> :O <sup>O</sup><br>152:0 |
| IL126-1C <sup>b</sup> | IL125-2B                             | C <sup>R</sup> E <sup>R</sup> :C <sup>O</sup> E <sup>O</sup> <sup>a</sup><br>31:22 | NT <sup>c</sup>                         |

<sup>a</sup> No other genotype found.

<sup>b</sup> Sum of petites induced after 320, 640, 960 and 1600 erg mm<sup>-2</sup> of UV.

<sup>c</sup> NT = Not tested.

of two types of crosses:  $\omega^+ \times \omega^+$  or  $\omega^- \times \omega^-$  which show no polarity are called homosexual crosses while  $\omega^+ \times \omega^-$  which display polarity are called heterosexual crosses. Studies on multifactorial crosses (Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974) have permitted to distinguish *polar* and *non-polar* regions of the mit-DNA molecules depending on their linkage or absence of linkage to the  $\omega$  locus.

The effects of UV will be described therefore separately on homosexual and on heterosexual crosses and in each case a distinction will be made between markers of the polar region and markers of the non polar regions.

#### a) Homosexual Two-Point Crosses

Different two-point homosexual crosses, both  $\omega^+ \times \omega^+$  and  $\omega^- \times \omega^-$ , have been performed with different configurations of the C and E alleles relatively to the cellular mating type.

In Table 3 each pair of haploid strains has been treated by UV in a symmetrical manner: either the  $\alpha$  parent has been irradiated and mated with a non irradiated  $\alpha$  parent or the  $\alpha$  parent has been irradiated and mated with a non irradiated  $\alpha$  parent.

In conformity with previous publications from this laboratory (Avner *et al.*, 1973, Wolf *et al.*, 1973, Netter *et al.*, 1974; Dujon *et al.*, 1974) and for reasons of convenience permitting a unified presentation of crosses involving haploids carrying different allelic configurations of mitochondrial genes, the transmission

Tables 3a and b. Effects of UV on two-point homosexual crosses

Table 3a

| Irradiated parent                                            | Non irradiated parent                               | UV dose<br>erg<br>mm <sup>-2</sup> | C <sup>a</sup> E <sup>a</sup> | C <sup>a</sup> E <sup>α</sup> | C <sup>α</sup> E <sup>a</sup> | C <sup>α</sup> E <sup>α</sup> | Total colonies | C <sup>a</sup> | E <sup>a</sup> |
|--------------------------------------------------------------|-----------------------------------------------------|------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------|----------------|----------------|
|                                                              |                                                     |                                    | C <sup>SER</sup>              | C <sup>SES</sup>              | C <sup>REER</sup>             | C <sup>RES</sup>              |                |                |                |
| IL187-7A<br>α<br>ω <sup>+</sup> C <sup>RES</sup><br>Ir. 4    | IL17-9B<br>a<br>ω <sup>+</sup> C <sup>SER</sup>     | 0                                  | 37.1                          | 6.7                           | 8.4                           | 47.8                          | 178            | 43.8           | 45.5           |
|                                                              |                                                     | 320                                | 51.0                          | 17.0                          | 5.2                           | 26.8                          | 153            | 68.0           | 56.2           |
|                                                              |                                                     | 640                                | 57.3                          | 7.0                           | 8.8                           | 26.9                          | 171            | 64.3           | 66.1           |
|                                                              |                                                     | 960                                | 65.5                          | 9.8                           | 8.0                           | 16.7                          | 174            | 75.3           | 73.5           |
|                                                              |                                                     | 1600                               | 73.0                          | 8.7                           | 2.6                           | 15.7                          | 115            | 81.7           | 75.6           |
| LM650-2C/36<br>α<br>ω <sup>+</sup> C <sup>RES</sup><br>Ir. 3 | IL17-4A<br>a<br>ω <sup>+</sup> C <sup>SER</sup>     | 0                                  | 67.2                          | 5.1                           | 1.6                           | 26.1                          | 314            | 72.3           | 68.8           |
|                                                              |                                                     | 640                                | 88.0                          | 3.5                           | 1.6                           | 7.0                           | 258            | 91.5           | 89.6           |
|                                                              |                                                     | 960                                | 87.3                          | 4.6                           | 0.5                           | 7.6                           | 197            | 91.9           | 87.8           |
|                                                              |                                                     | 1600                               | 94.7                          | 1.5                           | 0.5                           | 3.4                           | 206            | 96.2           | 95.2           |
|                                                              |                                                     |                                    |                               |                               |                               |                               |                |                |                |
| IL17-9B<br>a<br>ω <sup>+</sup> C <sup>SER</sup><br>Ir. 12    | IL187-7A<br>α<br>ω <sup>+</sup> C <sup>RES</sup>    | 0                                  | 35.2                          | 10.5                          | 6.8                           | 47.5                          | 219            | 45.7           | 42.0           |
|                                                              |                                                     | 320                                | 15.5                          | 6.3                           | 5.3                           | 72.9                          | 207            | 21.8           | 20.8           |
|                                                              |                                                     | 640                                | 12.6                          | 3.1                           | 4.7                           | 79.6                          | 191            | 15.7           | 17.3           |
|                                                              |                                                     | 960                                | 9.6                           | 3.5                           | 2.6                           | 84.2                          | 114            | 13.1           | 12.2           |
|                                                              |                                                     |                                    |                               |                               |                               |                               |                |                |                |
| IL17-4A<br>a<br>ω <sup>+</sup> C <sup>SER</sup><br>Ir. 11    | LM650-2C/36<br>α<br>ω <sup>+</sup> C <sup>RES</sup> | 0                                  | 48.6                          | 10.5                          | 4.4                           | 36.5                          | 181            | 59.1           | 53.0           |
|                                                              |                                                     | 320                                | 49.6                          | 9.2                           | 0.4                           | 40.8                          | 284            | 58.8           | 50.0           |
|                                                              |                                                     | 640                                | 35.8                          | 8.4                           | 1.3                           | 54.5                          | 299            | 44.2           | 37.1           |
|                                                              |                                                     | 960                                | 37.6                          | 7.8                           | 0.5                           | 54.1                          | 218            | 45.4           | 38.1           |
|                                                              |                                                     | 1600                               | 33.0                          | 6.2                           | 2.4                           | 58.4                          | 209            | 39.2           | 35.4           |

of alleles originated from the *a* parent is always given in the tables indistinctly whether the *a* parent is irradiated or whether the  $\alpha$  parent is irradiated. The adoption of this convention is not meant to imply any effect of the cellular mating type on the behavior of mitochondrial genes.

The Table 3 shows that when the  $\alpha$  parent is irradiated the transmission of C<sup>a</sup> and E<sup>a</sup> mitochondrial alleles diminishes, while when the *a* parent is irradiated the transmission of C<sup>a</sup> and E<sup>a</sup> increases (i.e. transmission of C<sup>α</sup> and E<sup>α</sup> alleles decreases). In all cases, both for  $\omega^+ \times \omega^+$  and  $\omega^- \times \omega^-$  crosses, UV irradiation diminishes the transmission into the cross of the alleles originated from the irradiated strain. The higher the UV dose is the lower the transmission of alleles of the irradiated parent and concomitantly the higher that of the other parent.

Fig. 2 shows the UV effects on transmission of alleles for two examples of homosexual crosses. This figure clearly points out that transmission of the same allele either increases or decreases depending on which parent is irradiated.

Avner *et al.* (1973), Wolf *et al.* (1973), Netter *et al.* (1974) have shown a co-ordinated transmission for all mitochondrial alleles in all homosexual crosses. It can be seen in Table 3 and on Fig. 2 that after any UV dose the transmission of the C allele is always similar to that of the E allele. This is true whatever the change in transmission induced by the UV irradiation is. In other words, in homo-

Table 3b

| Irradiated parent                                                          | Non irradiated parent                                            | UV dose<br>erg<br>mm <sup>-2</sup> | C <sup>a</sup> E <sup>a</sup> | C <sup>a</sup> E <sup>α</sup> | C <sup>α</sup> E <sup>a</sup> | C <sup>α</sup> E <sup>α</sup> | Total colonies | C <sup>a</sup> | E <sup>a</sup> |
|----------------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------|----------------|----------------|
|                                                                            |                                                                  |                                    | C <sup>R</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>S</sup> E <sup>R</sup> |                |                |                |
| 55R5-3C/321<br>a<br>ω <sup>-</sup> C <sup>R</sup> E <sup>S</sup><br>Ir. 25 | IL102-8D<br>α<br>ω <sup>-</sup> C <sup>S</sup> E <sup>R</sup>    | 0                                  | 49.3                          | 3.1                           | 6.9                           | 40.7                          | 290            | 52.4           | 56.2           |
|                                                                            |                                                                  | 320                                | 44.6                          | 2.7                           | 4.9                           | 47.8                          | 224            | 47.3           | 49.5           |
|                                                                            |                                                                  | 640                                | 50.6                          | 6.1                           | 4.1                           | 39.2                          | 245            | 56.7           | 54.7           |
|                                                                            |                                                                  | 960                                | 39.5                          | 3.4                           | 5.2                           | 51.9                          | 233            | 42.9           | 44.7           |
|                                                                            |                                                                  | 1600                               | 30.8                          | 2.7                           | 6.1                           | 60.3                          | 295            | 33.5           | 36.9           |
|                                                                            |                                                                  |                                    |                               |                               |                               |                               |                |                |                |
| IL16-10B<br>α<br>ω <sup>-</sup> C <sup>R</sup> E <sup>S</sup><br>Ir. 29    | 55R5-3C/221<br>a<br>ω <sup>-</sup> C <sup>S</sup> E <sup>R</sup> | 0                                  | 56.0                          | 2.2                           | 3.3                           | 38.6                          | 184            | 58.2           | 59.3           |
|                                                                            |                                                                  | 320                                | 53.1                          | 8.2                           | 4.1                           | 34.6                          | 243            | 61.3           | 57.2           |
|                                                                            |                                                                  | 640                                | 62.4                          | 5.6                           | 2.2                           | 29.8                          | 178            | 68.0           | 64.6           |
|                                                                            |                                                                  | 960                                | 64.8                          | 4.7                           | 4.0                           | 26.5                          | 253            | 69.5           | 68.8           |
|                                                                            |                                                                  | 1600                               | 79.4                          | 4.7                           | 2.9                           | 12.9                          | 170            | 84.1           | 82.3           |
|                                                                            |                                                                  |                                    |                               |                               |                               |                               |                |                |                |
| 55R5-3C/221<br>a<br>ω <sup>-</sup> C <sup>S</sup> E <sup>R</sup><br>Ir. 33 | IL16-10B<br>α<br>ω <sup>-</sup> C <sup>R</sup> E <sup>S</sup>    | 0                                  | 48.3                          | 7.3                           | 3.6                           | 40.7                          | 302            | 55.6           | 51.9           |
|                                                                            |                                                                  | 320                                | 50.4                          | 5.7                           | 1.4                           | 42.6                          | 141            | 56.1           | 51.8           |
|                                                                            |                                                                  | 640                                | 45.7                          | 6.8                           | 4.1                           | 43.4                          | 219            | 52.5           | 49.8           |
|                                                                            |                                                                  | 960                                | 41.4                          | 3.6                           | 4.1                           | 50.9                          | 169            | 45.0           | 45.5           |
|                                                                            |                                                                  | 1600                               | 32.4                          | 4.3                           | 4.8                           | 58.5                          | 188            | 36.7           | 37.2           |
|                                                                            |                                                                  |                                    |                               |                               |                               |                               |                |                |                |
| IL102-8D<br>α<br>ω <sup>-</sup> C <sup>S</sup> E <sup>R</sup><br>Ir. 34    | 55R5-3C/321<br>a<br>ω <sup>-</sup> C <sup>R</sup> E <sup>S</sup> | 0                                  | 46.6                          | 3.4                           | 8.5                           | 41.5                          | 176            | 50.0           | 55.1           |
|                                                                            |                                                                  | 320                                | 64.9                          | 3.2                           | 5.4                           | 26.6                          | 222            | 68.1           | 70.3           |
|                                                                            |                                                                  | 640                                | 65.2                          | 5.6                           | 4.5                           | 24.7                          | 178            | 70.8           | 69.7           |
|                                                                            |                                                                  | 960                                | 86.7                          | 3.3                           | 4.0                           | 6.0                           | 150            | 92.0           | 90.7           |
|                                                                            |                                                                  | 1600                               | 75.8                          | 3.8                           | 5.5                           | 14.8                          | 236            | 79.6           | 81.3           |

Quantitative random analysis by replica plating onto antibiotic containing media (N3, N3C, N3E, N3CE) of different two point homosexual crosses including the C<sup>R</sup><sub>321</sub>, C<sup>R</sup><sub>36</sub>, E<sup>R</sup><sub>221</sub> and E<sup>R</sup><sub>514</sub> mutations. Each row gives, for each dose of UV applied to one of the parents prior to mating, the frequency of the different classes (in %) amongst the diploids issued from the cross, the total number of colonies scored, and the frequency of the mitochondrial alleles originated from the *a* parent (in %).

Table 3a: ω<sup>+</sup> by ω<sup>+</sup> crosses; Table 3b: ω<sup>-</sup> by ω<sup>-</sup> crosses. Irradiation numbers (Ir.) refer to Tables 2a and 2b.

sexual crosses, both ω<sup>+</sup> × ω<sup>+</sup> and ω<sup>-</sup> × ω<sup>-</sup>, UV always affects similarly the C and the E alleles.

The degree of sensitivity to UV in respect to transmission of alleles (i.e. the relative change in transmission for a given dose of UV) varies from one experiment to another and within the same cross (between two given strains) depending on which of the parents is irradiated. This degree of sensitivity may be due on the one hand to the genotype of the irradiated strain and on the other hand to interactions between the two parents in the cross. However the fact that, in some crosses, the same UV dose when applied to one parent does not similarly modify the transmission of alleles as when applied to the other parent, favors

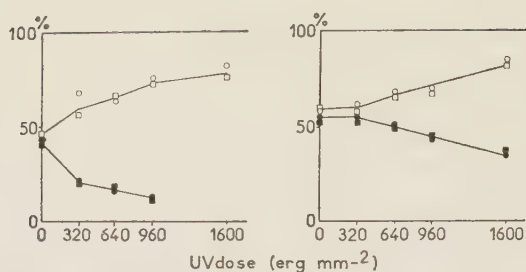


Fig. 2. Effect of UV irradiation applied to one or the other parent on transmission of mitochondrial alleles in homosexual crosses. Abscissae: UV dose in  $\text{erg mm}^{-2}$ . Ordinates: transmission of alleles originated from the  $a$  parent (in %). —●—, —■— respectively  $C^a$  and  $E^a$  alleles when the  $a$  parent is irradiated. —○—, —□— respectively  $C^a$  and  $E^a$  alleles when the  $\alpha$  parent is irradiated. Left diagram: IL17-9B ( $a$ ,  $\omega^+$   $C^{iS^E R}$ )  $\times$  IL187-7A ( $a$ ,  $\omega^+$   $C^{E^i S}$ ). Right diagram: 55R5-3C/221 ( $a$ ,  $\omega^-$   $C^{iS^E R}$ )  $\times$  IL16-10B ( $a$ ,  $\omega^-$   $C^{E^i S}$ ). Examples taken from Table 3.

the idea that the degree of sensitivity is mainly due to the genotype of the strain which is irradiated. For example in the cross 55R5-3C/321  $\times$  IL102-8D, a UV dose of 640  $\text{erg mm}^{-2}$  applied to IL102-8D changes the transmission of the  $C^a$  and  $E^a$  alleles respectively from 50.0% to 70.8% and from 55.1% to 69.7%, while the same UV dose applied to 55R5-3C/321 practically does not modify the transmission of the same alleles (respectively 52.4% to 56.7% and 56.2% to 54.7%). Furthermore, the fact that two strains with the same mitochondrial genotypes (for examples IL17-4A and IL17-9B) but with different nuclear genotypes have different degrees of sensitivity to UV for transmission of mitochondrial alleles in crosses, show that the degree of sensitivity is mainly due to the *nuclear* genotype of the strain which is irradiated. Finally it should be noted that this degree of sensitivity is neither correlated to the cellular mating type ( $a/\alpha$ ) of the irradiated strain nor to the mitochondrial alleles it carries. The degree of sensitivity will be studied in relationship to cell survival and petite induction in the Discussion.

Effects of UV irradiation on the overall frequency of recombinants between the C and E markers is more difficult to study than the effects on transmission, since the frequency of recombinants between these two markers is relatively low and the sampling error becomes very important. These effects will be studied in the next paragraph.

However it clearly appears from Table 3 that polarity of recombination is not significantly modified after UV irradiation i.e. the two types of recombinants have, within sampling errors, similar frequencies. This is true for both  $\omega^+ \times \omega^+$  and  $\omega^- \times \omega^-$  crosses.

In conclusion, in homosexual crosses, UV irradiation of one of the parents prior to mating considerably diminishes the transmission of the mitochondrial alleles brought into the cross by the irradiated parent. It does not modify significantly the polarity of recombination.

#### b) Homosexual Three-Point Crosses

Table 4 shows the results of three different three-point homosexual crosses involving markers of the polar region (C and E) and markers of non polar regions (O and P).

Like in the case of two-point crosses described before, in all three-point crosses the transmission of mitochondrial alleles of the irradiated parent is diminished by increasing doses of UV. This diminution exists for all the markers studied so far, both those located in the polar region (C and E) and those located in non polar regions (O and P). Furthermore, in all crosses and after any UV dose, the transmission of all the mitochondrial alleles of the same parent is always similarly affected (compare  $C^a$ ,  $E^a$  and  $O^a$  or  $C^a$ ,  $E^a$  and  $P^a$ , or  $C^a$ ,  $O^a$  and  $P^a$ ). In other words, in homosexual crosses the rule of *coordinated transmission* holds true for all the markers and whatever the change in transmission induced by the UV irradiation. We have discussed at length in the preceding paragraph arguments in favour of the idea that the sensitivity to UV as measured by the decrease in transmission of mitochondrial alleles is mainly due to the nuclear genetic background. Results of three point crosses support this idea further. There are some fluctuations in the results of repeats of the same cross, whether irradiated or not. These fluctuations can be partially accounted for by the sampling error of diploid colonies scored but also can be due to other sources of variations, which are more difficult to assess, such as the total number of zygotic colonies harvested to constitute the population of diploid cells, variations in physiological conditions of the irradiated cells etc.

Effects of UV irradiation on the overall frequency of recombinants can be studied in more details in three point crosses than in two points ones since six recombinant classes exist instead of two, and double recombination events can be studied. Table 4 shows that the overall frequency of recombinants between two markers may significantly diminish when high UV doses are applied to one of the parents. For example in the cross DP1-1B/7  $\times$  IL8-8D the total frequency of recombinants between C and O markers are respectively 16.1% and 14.2% for control crosses (experiments Ir 16 and Ir 18) while after a UV dose of 1600 ergs there are only 3.4% and 2.3% of the same recombinants. A similar diminution of the overall frequency of recombinants after high UV doses can be seen between C and P markers (experiments Ir 35 and Ir 37) where there are respectively 9.0 and 16.4% of recombinants in control crosses and only 3.0 and 7.4% after 1600 ergs of UV. Similar diminution of the overall frequency of recombinants between E and O markers and E and P markers are also visible. For the CE pair of markers the total frequency of recombinants is much lower for the cross M12/54  $\times$  PS189-3A than for other crosses (see the Discussion). The cross DP1-1B/7  $\times$  IL8-8D shows that high UV doses also diminish the total frequency of recombinants between the C and E markers.

In all cases low UV doses (e.g. 320 or 640 ergs) and in some experiments (Ir 17) even a high UV dose do not significantly diminish the frequency of recombinants. As we shall see in Discussion there is a correlation between the frequency of recombinants and the bias in transmission of alleles: the higher the bias in transmission, the lower the frequency of recombinants between two loci. The table shows that when transmission of alleles is strongly biased (i.e. greater than 90% or smaller than 10%) the frequency of recombinants is significantly diminished, while in cases where the bias in transmission is not so important little or no diminution of the frequency of recombinants is observed.

In homosexual crosses no polarity of recombination is observed for any pair of markers. After UV irradiation fluctuations of the polarity within the limits

Table 4. Effects of UV on

| Irradiated parent                                           | Non irradiated parent                                       | UV dose<br>erg<br>mm <sup>-2</sup> | C <sup>a</sup> E <sup>a</sup> O <sup>a</sup> | C <sup>a</sup> E <sup>a</sup> O <sup>α</sup> | C <sup>a</sup> E <sup>α</sup> O <sup>a</sup> | C <sup>a</sup> E <sup>α</sup> O <sup>α</sup> | C <sup>α</sup> E <sup>a</sup> O <sup>a</sup> |
|-------------------------------------------------------------|-------------------------------------------------------------|------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|----------------------------------------------|
|                                                             |                                                             |                                    | C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> O <sup>R</sup> | C <sup>R</sup> E <sup>S</sup> O <sup>S</sup> | C <sup>R</sup> E <sup>S</sup> O <sup>R</sup> | C <sup>S</sup> E <sup>R</sup> O <sup>S</sup> |
| DP1-1B/7                                                    | IL8-8D                                                      | 0                                  | 32.4                                         | 1.9                                          | 2.9                                          | 4.3                                          | 5.3                                          |
|                                                             |                                                             | 320                                | 52.6                                         | 6.2                                          | 2.6                                          | 2.2                                          | 4.7                                          |
| α                                                           | a                                                           | 640                                | 56.8                                         | 5.9                                          | 0.0                                          | 2.7                                          | 3.2                                          |
| ω <sup>+</sup> C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> | ω <sup>+</sup> C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | 960                                | 54.5                                         | 8.6                                          | 2.1                                          | 2.4                                          | 3.4                                          |
| Ir. 17                                                      |                                                             | 1600                               | 52.1                                         | 7.7                                          | 1.8                                          | 0.6                                          | 3.6                                          |
| DP1-1B/7                                                    | IL8-8D                                                      | 0                                  | 33.0                                         | 4.2                                          | 1.9                                          | 1.4                                          | 6.6                                          |
|                                                             |                                                             | 320                                | 50.0                                         | 4.1                                          | 0.8                                          | 3.3                                          | 4.1                                          |
| α                                                           | a                                                           | 640                                | 59.3                                         | 7.1                                          | 1.3                                          | 2.4                                          | 2.0                                          |
| ω <sup>+</sup> C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> | ω <sup>+</sup> C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | 960                                | 76.5                                         | 3.0                                          | 1.8                                          | 1.8                                          | 1.8                                          |
| Ir. 16                                                      |                                                             | 1600                               | 89.9                                         | 2.4                                          | 1.0                                          | 0.0                                          | 0.0                                          |
| DP1-1B/7                                                    | IL8-8D                                                      | 0                                  | 50.5                                         | 4.1                                          | 3.7                                          | 1.8                                          | 3.5                                          |
| α                                                           | a                                                           | 640                                | 77.8                                         | 3.5                                          | 2.6                                          | 0.9                                          | 2.4                                          |
| ω <sup>+</sup> C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> | ω <sup>+</sup> C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | 960                                | 89.4                                         | 2.2                                          | 1.1                                          | 0.4                                          | 1.0                                          |
| Ir. 18                                                      |                                                             | 1600                               | 94.7                                         | 0.9                                          | 0.8                                          | 0.1                                          | 0.9                                          |
| IL8-8D                                                      | DP1-1B/7                                                    | 0                                  | 50.9                                         | 4.0                                          | 4.0                                          | 2.5                                          | 5.1                                          |
|                                                             |                                                             | 1600                               | 16.3                                         | 1.6                                          | 3.4                                          | 1.4                                          | 3.7                                          |
| a                                                           | α                                                           | 1760                               | 25.2                                         | 2.5                                          | 1.8                                          | 1.9                                          | 4.9                                          |
| ω <sup>+</sup> C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | ω <sup>+</sup> C <sup>S</sup> E <sup>S</sup> O <sup>R</sup> | 1920                               | 20.7                                         | 2.1                                          | 2.7                                          | 1.1                                          | 2.7                                          |
| Ir. 22                                                      |                                                             | 2080                               | 23.4                                         | 2.4                                          | 2.9                                          | 1.6                                          | 4.2                                          |
|                                                             |                                                             |                                    | C <sup>a</sup> E <sup>a</sup> P <sup>a</sup> | C <sup>a</sup> E <sup>a</sup> P <sup>α</sup> | C <sup>a</sup> E <sup>α</sup> P <sup>a</sup> | C <sup>a</sup> E <sup>α</sup> P <sup>α</sup> | C <sup>α</sup> E <sup>a</sup> P <sup>a</sup> |
|                                                             |                                                             |                                    | C <sup>S</sup> E <sup>S</sup> P <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> P <sup>S</sup> | C <sup>S</sup> E <sup>R</sup> P <sup>R</sup> | C <sup>S</sup> E <sup>R</sup> P <sup>S</sup> | C <sup>R</sup> E <sup>S</sup> P <sup>R</sup> |
| M12/54                                                      | PS189-3A                                                    | 0                                  | 27.7                                         | 5.0                                          | 0.5                                          | 0.2                                          | 0.2                                          |
| a                                                           | α                                                           | 480                                | 8.7                                          | 4.2                                          | 0.1                                          | 0.9                                          | 0.2                                          |
| ω <sup>-</sup> C <sup>S</sup> E <sup>S</sup> P <sup>R</sup> | ω <sup>-</sup> C <sup>R</sup> E <sup>R</sup> P <sup>S</sup> | 960                                | 5.3                                          | 3.0                                          | 0.2                                          | 0.6                                          | 0.4                                          |
| Ir. 35                                                      |                                                             | 1600                               | 0.6                                          | 1.0                                          | 0.1                                          | 0.3                                          | 0.2                                          |
| PS189-3A                                                    | M12/54                                                      | 0                                  | 51.2                                         | 9.3                                          | 0.4                                          | 0.1                                          | 1.3                                          |
| α                                                           | a                                                           | 480                                | 65.1                                         | 7.7                                          | 0.2                                          | 0.1                                          | 0.9                                          |
| ω <sup>-</sup> C <sup>R</sup> E <sup>R</sup> P <sup>S</sup> | ω <sup>-</sup> C <sup>S</sup> E <sup>S</sup> P <sup>R</sup> | 960                                | 79.1                                         | 5.3                                          | 0.5                                          | 0.3                                          | 0.6                                          |
| Ir. 37                                                      |                                                             | 1600                               | 91.2                                         | 7.2                                          | 0.2                                          | 0.1                                          | 0.0                                          |
|                                                             |                                                             |                                    | C <sup>a</sup> O <sup>a</sup> P <sup>a</sup> | C <sup>a</sup> O <sup>a</sup> P <sup>α</sup> | C <sup>a</sup> O <sup>α</sup> P <sup>a</sup> | C <sup>a</sup> O <sup>α</sup> P <sup>α</sup> | C <sup>α</sup> O <sup>a</sup> P <sup>a</sup> |
|                                                             |                                                             |                                    | C <sup>R</sup> O <sup>R</sup> P <sup>R</sup> | C <sup>R</sup> O <sup>R</sup> P <sup>S</sup> | C <sup>R</sup> O <sup>S</sup> P <sup>R</sup> | C <sup>R</sup> O <sup>S</sup> P <sup>S</sup> | C <sup>S</sup> O <sup>R</sup> P <sup>R</sup> |
| KL14-4A                                                     | IL216-1B                                                    | 0                                  | 23.3                                         | 3.4                                          | 6.9                                          | 4.4                                          | 4.7                                          |
| a                                                           | α                                                           | 1600                               | 12.1                                         | 2.9                                          | 5.2                                          | 3.9                                          | 4.3                                          |
| ω <sup>+</sup> C <sup>R</sup> O <sup>R</sup> P <sup>R</sup> | ω <sup>+</sup> C <sup>S</sup> O <sup>S</sup> P <sup>S</sup> |                                    |                                              |                                              |                                              |                                              |                                              |
| Ir. 24 <sup>a</sup>                                         |                                                             |                                    |                                              |                                              |                                              |                                              |                                              |

<sup>a</sup> E<sup>R</sup> versus E<sup>S</sup> phenotypes have not been scored.

Quantitative random analysis by replica plating onto antibiotic containing media (N3, N3C, N3E, N3O, N3CE, N3CO, K3P, K3OP, according to the markers present in the cross) of different three point homosexual crosses including the C<sub>321</sub><sup>R</sup>, E<sub>221</sub><sup>R</sup>, E<sub>514</sub><sup>R</sup>, O<sub>1</sub><sup>R</sup>, O<sub>7</sub><sup>R</sup> and P<sub>454</sub><sup>R</sup>

## three-point homosexual crosses

| $C^{\alpha}E^{\alpha}O^{\alpha}$ | $C^{\alpha}E^{\alpha}O^a$        | $C^{\alpha}E^{\alpha}O^{\alpha}$ | Total colonies | $C^a$ | $E^a$ | $O^a$ | $C^{\alpha}E^{\alpha}/C^{\alpha}E^a$ | $C^{\alpha}O^{\alpha}/C^{\alpha}O^a$ | $E^{\alpha}O^{\alpha}/E^{\alpha}O^a$ |
|----------------------------------|----------------------------------|----------------------------------|----------------|-------|-------|-------|--------------------------------------|--------------------------------------|--------------------------------------|
| $C^{\alpha}E^{\alpha}O^{\alpha}$ | $C^{\alpha}E^{\alpha}O^{\alpha}$ | $C^{\alpha}E^{\alpha}O^{\alpha}$ |                |       |       |       |                                      |                                      |                                      |
| 3.9                              | 3.9                              | 45.4                             | 207            | 41.5  | 43.5  | 44.4  | 7.2/8.2                              | 6.3/9.2                              | 5.8/6.8                              |
| 5.5                              | 2.6                              | 23.7                             | 274            | 63.5  | 69.0  | 62.4  | 4.7/10.2                             | 8.4/7.3                              | 11.7/5.1                             |
| 3.6                              | 4.5                              | 23.4                             | 222            | 65.3  | 69.4  | 64.4  | 2.7/6.8                              | 8.6/7.7                              | 9.5/4.5                              |
| 4.1                              | 3.4                              | 21.6                             | 292            | 67.5  | 70.5  | 63.4  | 4.4/7.5                              | 11.0/6.8                             | 12.7/5.5                             |
| 4.7                              | 4.7                              | 24.9                             | 169            | 62.1  | 68.0  | 62.1  | 2.4/8.3                              | 8.3/8.3                              | 12.4/6.5                             |
| 1.9                              | 3.8                              | 47.2                             | 212            | 40.6  | 45.8  | 45.3  | 3.3/8.5                              | 5.7/10.4                             | 6.1/5.7                              |
| 3.3                              | 4.5                              | 29.9                             | 244            | 58.2  | 61.5  | 59.4  | 4.1/7.4                              | 7.4/8.6                              | 7.4/5.3                              |
| 2.7                              | 4.0                              | 21.2                             | 297            | 70.0  | 71.0  | 66.7  | 3.7/4.7                              | 9.4/6.1                              | 9.8/5.4                              |
| 3.0                              | 2.4                              | 9.6                              | 166            | 83.1  | 84.3  | 82.5  | 3.6/4.8                              | 4.8/4.2                              | 6.0/4.2                              |
| 0.5                              | 1.0                              | 5.3                              | 207            | 93.2  | 92.8  | 91.8  | 1.0/0.5                              | 2.4/1.0                              | 2.9/1.9                              |
| 3.0                              | 4.8                              | 28.5                             | 2158           | 60.1  | 61.2  | 62.5  | 5.5/6.5                              | 5.9/8.3                              | 7.2/8.5                              |
| 2.4                              | 1.5                              | 8.9                              | 541            | 84.8  | 86.1  | 84.3  | 3.5/4.8                              | 4.4/3.9                              | 5.9/4.1                              |
| 0.7                              | 0.9                              | 4.4                              | 1024           | 93.1  | 93.3  | 92.3  | 1.5/1.7                              | 2.6/1.9                              | 2.9/1.9                              |
| 0.2                              | 0.4                              | 2.1                              | 1159           | 96.5  | 96.6  | 96.7  | 0.9/1.0                              | 1.0/1.3                              | 1.1/1.2                              |
| 3.0                              | 5.8                              | 24.8                             | 605            | 61.3  | 63.0  | 65.8  | 6.4/8.1                              | 6.4/10.9                             | 6.9/9.7                              |
| 3.2                              | 7.4                              | 63.2                             | 1013           | 22.6  | 24.7  | 30.7  | 4.7/6.8                              | 2.3/11.1                             | 4.7/10.8                             |
| 3.0                              | 6.3                              | 54.5                             | 734            | 31.3  | 35.6  | 38.1  | 3.7/7.9                              | 4.4/11.2                             | 5.4/8.0                              |
| 2.1                              | 7.6                              | 60.9                             | 990            | 26.7  | 27.7  | 33.7  | 3.8/4.8                              | 3.2/10.3                             | 4.2/10.3                             |
| 2.0                              | 7.8                              | 55.7                             | 887            | 30.3  | 32.0  | 38.3  | 4.5/6.2                              | 3.9/11.9                             | 4.4/10.7                             |
| $C^{\alpha}E^{\alpha}P^{\alpha}$ | $C^{\alpha}E^{\alpha}P^a$        | $C^{\alpha}E^{\alpha}P^{\alpha}$ |                | $C^a$ | $E^a$ | $P^a$ | $C^{\alpha}E^{\alpha}/C^{\alpha}E^a$ | $C^{\alpha}P^{\alpha}/C^{\alpha}P^a$ | $E^{\alpha}P^{\alpha}/E^{\alpha}P^a$ |
| $C^{\alpha}E^{\alpha}P^{\alpha}$ | $C^{\alpha}E^{\alpha}P^{\alpha}$ | $C^{\alpha}E^{\alpha}P^{\alpha}$ |                |       |       |       |                                      |                                      |                                      |
| 0.9                              | 3.6                              | 61.8                             | 555            | 33.5  | 33.9  | 32.1  | 0.7/1.1                              | 5.2/3.8                              | 5.9/4.1                              |
| 1.0                              | 2.8                              | 82.1                             | 814            | 13.9  | 14.1  | 11.9  | 1.0/1.2                              | 5.0/3.1                              | 5.2/2.9                              |
| 0.8                              | 3.1                              | 86.7                             | 908            | 9.0   | 9.5   | 9.0   | 0.8/1.2                              | 3.5/3.5                              | 3.7/3.3                              |
| 0.4                              | 1.6                              | 95.8                             | 1117           | 2.0   | 2.2   | 2.5   | 0.4/0.6                              | 1.2/1.8                              | 1.4/1.7                              |
| 0.8                              | 5.7                              | 31.3                             | 774            | 61.0  | 62.5  | 58.5  | 0.5/2.1                              | 9.4/7.0                              | 10.1/6.1                             |
| 0.5                              | 4.8                              | 20.7                             | 816            | 73.2  | 74.1  | 71.0  | 0.4/1.3                              | 7.8/5.6                              | 8.2/5.0                              |
| 0.4                              | 3.3                              | 10.3                             | 939            | 85.3  | 85.5  | 83.6  | 0.8/1.1                              | 5.6/3.9                              | 5.7/3.8                              |
| 0.0                              | 0.1                              | 1.2                              | 1107           | 98.7  | 98.5  | 91.5  | 0.3/0.0                              | 7.3/0.1                              | 7.2/0.3                              |
| $C^{\alpha}O^{\alpha}P^{\alpha}$ | $C^{\alpha}O^{\alpha}P^a$        | $C^{\alpha}O^{\alpha}P^{\alpha}$ |                | $C^a$ | $O^a$ | $P^a$ | $C^{\alpha}O^{\alpha}/C^{\alpha}O^a$ | $C^{\alpha}P^{\alpha}/C^{\alpha}P^a$ | $O^{\alpha}P^{\alpha}/O^{\alpha}P^a$ |
| $C^{\alpha}O^{\alpha}P^{\alpha}$ | $C^{\alpha}O^{\alpha}P^{\alpha}$ | $C^{\alpha}O^{\alpha}P^{\alpha}$ |                |       |       |       |                                      |                                      |                                      |
| 6.7                              | 5.3                              | 45.2                             | 1059           | 38.1  | 38.1  | 40.2  | 11.3/11.4                            | 7.8/10.0                             | 10.1/12.2                            |
| 4.2                              | 6.8                              | 60.6                             | 763            | 24.1  | 23.5  | 28.4  | 9.2/8.5                              | 6.8/11.1                             | 7.1/12.1                             |

mutations. Each row gives, for each dose of UV applied to one of the parents prior to mating, the frequency of the different classes (in %) amongst the diploids issued from the cross, the total number of colonies scored, and the frequency of the mitochondrial alleles originated from the  $a$  parent (in %).

Irradiation numbers (Ir.) refer to Tables 2a and b.

of sampling error are observed but polarity of recombination is not highly modified. This is true whatever the diminution of the overall frequency of recombinants introduced by UV. However some bias can be observed in some cases. For example on the cross PS189-3A irradi.  $\times$  M12/54 there are respectively 7.3% versus 0.1% of  $C^+P^+$  and  $C^+P^-$  recombinants and 7.2% versus 0.3% of  $E^+P^+$  and  $E^+P^-$  recombinants. As will be discussed later this bias may be interpreted in terms of petite induction and should be distinguished from polarity of recombination due to the  $\omega$  locus.

In conclusion, in homosexual crosses, UV irradiation of one parent diminishes similarly the transmission of all the alleles of the irradiated parent and diminishes the overall frequency of recombinants when the transmission is highly biased. Polarity of recombination remains unchanged.

### c) Heterosexual Two-Point Crosses

Various two-point heterosexual crosses have been performed with different configurations of the C and E alleles relative to the cellular mating type and to the  $\omega$  locus. In each case either the  $\omega^+$  parent has been irradiated and mated to a non irradiated  $\omega^-$  parent or the  $\omega^-$  parent has been irradiated and mated to a non irradiated  $\omega^+$  parent. In conformity with previous publications from this laboratory (Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974; Dujon *et al.*, 1974) and for reasons of convenience permitting a unified presentation of crosses involving haploids carrying different allelic configurations of mitochondrial genes the transmission of alleles originated from the  $\omega^+$  parent is always given in tables indistinctly of whether the  $\omega^+$  parent or the  $\omega^-$  parent is irradiated.

Table 5a shows the results of irradiation of the  $\omega^+$  parents.

In all cases the transmission of the alleles carried by the  $\omega^+$  parent diminishes as a function of UV dose. However for the same UV dose the relative diminution of transmission of the  $E^+$  allele ( $R_{III}$  locus) is always more important than that of the  $C^+$  allele ( $R_I$  locus). In other words in heterosexual crosses UV irradiation of the  $\omega^+$  parent increases the difference in transmission between the  $C^+$  and the  $E^+$  allele observed for control crosses since the  $C^+$  allele which shows the highest transmission is the less affected by UV.

The relative diminution of transmission of the  $E^+$  allele at the  $R_{II}$  locus is in between that of the  $C^+$  allele ( $R_I$  locus) and that of the  $E^+$  allele at the  $R_{III}$  locus. In heterosexual crosses there is a differential decrease of transmission of mitochondrial alleles after UV.

The frequency of the majority class recombinants ( $C^+E^-$ ) is significantly increased by UV irradiation of the  $\omega^+$  parent. In most cases the maximum frequency of  $C^+E^-$  recombinants (around 50% to 60% in average for  $R_I$  and  $R_{III}$  loci and around 20% for  $R_I$  and  $R_{II}$  loci) is obtained for UV doses of 640 and 960 ergs and not for the highest UV dose (1600 ergs). As the frequency of the minority class recombinants is very low and the sampling error is considerable no valuable information of the UV effects on this class can be obtained by the replica plating scoring of the total population. However, it is clear that no major modification of the polarity of recombination is observed i.e. after UV the polarity of recombination between the C and E marker is always very high and is never comparable to that observed in homosexual crosses. This conclusion is confirmed by the

analysis of the minority class recombinants by the aliquot plating technique on selective media (see Avner *et al.*, 1973, Table 15 for the procedure). The frequency of C-E<sup>+</sup> recombinants after UV irradiation remains similar or increases at most by a factor of 2 (data not shown). The polarity of recombination is therefore not greatly modified although some minor changes are not excluded.

Table 5b shows the results of irradiation of the  $\omega^-$  parents.

In all cases the transmission of the C<sup>+</sup> and E<sup>+</sup> alleles increases as a function of UV dose i.e. transmission of the alleles brought into the cross by the irradiated parent (C<sup>-</sup> and E<sup>-</sup>) decreases.

The frequency of the major recombinant class (C<sup>+</sup>E<sup>-</sup>) is diminished after UV irradiation. The higher the UV dose the lower the frequency of C<sup>+</sup>E<sup>-</sup> recombinants. Effect of UV on the minor class recombinant (C<sup>-</sup>E<sup>+</sup>) is difficult to study since the frequency of this recombinant class is too low and sampling error becomes very important. However it is clear that the polarity of recombination is not greatly changed by UV.

In conclusion, in heterosexual crosses there is a striking difference between the effects of UV applied to the  $\omega^+$  parent and the effects of UV applied to the  $\omega^-$  parent. In both cases the transmission of the alleles of the irradiated parent diminishes but in the first case UV *increases* the frequency of the major recombinant class up to a maximum of 50–60% while in the second case it *decreases* the frequency of the same class. In all cases the polarity of recombination between C and E remains very high.

#### d) Heterosexual Three-Point Crosses

Table 6 shows the results of different three points (C, E, O) heterosexual crosses.

In all cases where the  $\omega^+$  parent is irradiated the transmission of C<sup>+</sup>, E<sup>+</sup> and O<sup>+</sup> alleles diminishes with increasing UV doses. However for the same UV dose the relative diminution of transmission is more pronounced for the O<sup>+</sup> allele than it is for the E<sup>+</sup> allele and than it is for the C<sup>+</sup> one: there is a *differential decrease* of transmission of the mitochondrial markers after UV irradiation of the  $\omega^+$  parent. The decrease of transmission by UV is correlated with the transmission of the mitochondrial alleles of the  $\omega^+$  parent in control crosses: the higher is the transmission of the allele, the smaller the relative decrease induced by UV. Transmission of alleles of mitochondrial loci in non polar regions (such as O) is highly affected by UV while that of alleles of the polar region (such as E and C) is less affected. In conclusion the closer a marker is to  $\omega$  the less diminished is the transmission of its allele brought into the cross by the irradiated  $\omega^+$  parent.

In the cross IL126-1C irr.  $\times$  DP1-1B/7 where the  $\omega^-$  parent is irradiated no significant changes of transmission of alleles are observed. This may be due to the fact that the strain IL126-1C has a small sensitivity to UV for transmission of alleles and not to the fact that it is  $\omega^-$  since, in Table 5, irradiation of other  $\omega^-$  parent showed a small decrease of transmission of alleles.

In heterosexual crosses effects of UV on the total frequency of recombinants depends on the pair of markers considered. Between C and O markers the majority class recombinant (C<sup>+</sup>O<sup>-</sup>) is significantly increased by UV irradiation of the  $\omega^+$  parent. The maximum frequency of C<sup>+</sup>O<sup>-</sup> recombinants reaches values as high as 80% i.e. in these cases the progeny of the cross is composed in majority of one recombinant class. The same conclusions apply to the major recombinant

Tables 5 a and b. Effects of UV on heterosexual two-point crosses  
Table 5a

| Irradiated parent                        | Non irradiated parent                    | UV dose<br>erg<br>mm <sup>-2</sup> | C+E <sup>+</sup>              | C+E <sup>-</sup>              | C-E <sup>+</sup>              | C-E <sup>-</sup>              | Total colonies | C <sup>+</sup> | E <sup>+</sup> |
|------------------------------------------|------------------------------------------|------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------|----------------|----------------|
|                                          |                                          |                                    | C <sup>S</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>R</sup> E <sup>S</sup> |                |                |                |
| DP1-1B/514                               | 55R5-3C/321                              | 0                                  | 60.8                          | 39.2                          | 0.0                           | 0.0                           | 268            | 100            | 60.8           |
| $\alpha$                                 | a                                        | 320                                | 40.5                          | 49.5                          | 1.8                           | 8.1                           | 222            | 90.0           | 42.3           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 640                                | 19.1                          | 60.8                          | 0.0                           | 20.1                          | 194            | 79.9           | 19.1           |
| Ir. 7                                    |                                          | 960                                | 18.1                          | 44.0                          | 0.9                           | 37.1                          | 232            | 62.1           | 19.0           |
| DP1-1B/514                               | 55R5-3C/321                              | 0                                  | 73.0                          | 25.8                          | 0.0                           | 1.1                           | 267            | 98.8           | 73.0           |
| $\alpha$                                 | a                                        | 640                                | 44.4                          | 47.8                          | 1.3                           | 6.5                           | 232            | 92.2           | 45.7           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 960                                | 26.5                          | 59.6                          | 0.0                           | 13.9                          | 245            | 80.1           | 26.5           |
| Ir. 8                                    |                                          | 1600                               | 20.3                          | 49.1                          | 0.5                           | 30.2                          | 212            | 69.4           | 20.8           |
| DP1-1B/514                               | 55R5-3C/321                              | 0                                  | 85.1                          | 14.9                          | 0.0                           | 0.0                           | 242            | 100            | 85.1           |
| $\alpha$                                 | a                                        | 320                                | 52.1                          | 41.5                          | 0.0                           | 6.4                           | 188            | 93.6           | 52.1           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 640                                | 47.0                          | 42.1                          | 1.6                           | 9.3                           | 183            | 89.1           | 48.6           |
| Ir. 6                                    |                                          | 960                                | 36.4                          | 49.1                          | 1.3                           | 13.2                          | 448            | 85.5           | 37.7           |
|                                          |                                          | 1600                               | 17.4                          | 39.8                          | 0.0                           | 42.8                          | 201            | 57.2           | 17.4           |
| DP1-1B/514                               | IL16-11D                                 | 0                                  | 80.0                          | 19.5                          | 0.0                           | 0.5                           | 190            | 99.5           | 80.0           |
| $\alpha$                                 | a                                        | 320                                | 71.6                          | 26.7                          | 0.0                           | 1.7                           | 232            | 98.3           | 71.6           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 640                                | 63.7                          | 34.3                          | 0.4                           | 1.6                           | 245            | 98.0           | 64.1           |
| Ir. 9                                    |                                          | 960                                | 59.5                          | 35.5                          | 0.0                           | 5.0                           | 200            | 95.0           | 59.5           |
|                                          |                                          | 1600                               | 30.4                          | 40.4                          | 0.0                           | 29.2                          | 171            | 70.8           | 30.4           |
| IL17-1D                                  | 55R5-3C/321                              | 0                                  | 82.4                          | 16.7                          | 0.0                           | 0.9                           | 221            | 99.1           | 82.4           |
| $\alpha$                                 | a                                        | 320                                | 59.6                          | 37.4                          | 0.0                           | 3.0                           | 198            | 97.0           | 59.6           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 640                                | 47.9                          | 44.0                          | 0.0                           | 8.1                           | 234            | 91.9           | 47.9           |
| Ir. 10                                   |                                          | 960                                | 35.2                          | 56.8                          | 1.1                           | 6.8                           | 176            | 92.0           | 36.3           |
|                                          |                                          | 1600                               | 18.2                          | 55.0                          | 1.2                           | 25.6                          | 242            | 73.2           | 19.4           |
| IL17-9B                                  | IL16-10B                                 | 0                                  | 68.6                          | 30.0                          | 0.0                           | 1.4                           | 201            | 98.6           | 68.6           |
| a                                        | $\alpha$                                 | 320                                | 61.6                          | 32.7                          | 0.0                           | 5.7                           | 245            | 94.3           | 61.6           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 640                                | 50.7                          | 42.6                          | 0.0                           | 6.7                           | 209            | 93.3           | 50.7           |
| Ir. 13                                   |                                          |                                    |                               |                               |                               |                               |                |                |                |
| IL17-9B                                  | IL16-10B                                 | 0                                  | 79.2                          | 19.0                          | 0.0                           | 1.8                           | 226            | 98.2           | 79.2           |
| a                                        | $\alpha$                                 | 320                                | 62.9                          | 35.1                          | 0.5                           | 1.5                           | 205            | 98.0           | 63.4           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 640                                | 48.0                          | 40.5                          | 0.5                           | 11.0                          | 200            | 88.5           | 48.5           |
| Ir. 14                                   |                                          | 960                                | 38.2                          | 51.4                          | 0.0                           | 10.4                          | 173            | 89.6           | 38.2           |
|                                          |                                          |                                    |                               |                               |                               |                               |                |                |                |
|                                          |                                          |                                    | C <sup>R</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>S</sup> E <sup>R</sup> |                |                |                |
| LM650-2C/36                              | 55R5-3C/221                              | 0                                  | 85.3                          | 9.8                           | 0.9                           | 4.0                           | 225            | 95.1           | 86.2           |
| $\alpha$                                 | a                                        | 640                                | 41.8                          | 46.9                          | 0.0                           | 11.3                          | 275            | 88.7           | 41.8           |
| $\omega^+$ C <sup>R</sup> E <sup>S</sup> | $\omega^-$ C <sup>S</sup> E <sup>R</sup> | 960                                | 25.9                          | 48.7                          | 0.0                           | 25.4                          | 193            | 74.6           | 25.9           |
| Ir. 1                                    |                                          | 1600                               | 20.6                          | 40.0                          | 0.6                           | 38.7                          | 155            | 60.6           | 21.2           |
| LM650-2C/36                              | 55R5-3C/221                              | 0                                  | 90.8                          | 8.8                           | 0.0                           | 0.4                           | 240            | 99.6           | 90.8           |
| $\alpha$                                 | a                                        | 640                                | 45.2                          | 40.2                          | 0.0                           | 14.6                          | 219            | 85.4           | 45.2           |
| $\omega^+$ C <sup>R</sup> E <sup>S</sup> | $\omega^-$ C <sup>S</sup> E <sup>R</sup> | 960                                | 28.7                          | 48.3                          | 0.0                           | 23.0                          | 230            | 77.0           | 28.7           |
| Ir. 2                                    |                                          | 1600                               | 12.3                          | 50.9                          | 0.0                           | 36.8                          | 269            | 63.2           | 12.3           |

Table 5a (continued)

| Irradiated parent                        | Non irradiated parent                    | UV dose erg mm <sup>-2</sup> | C <sup>+</sup> E <sup>+</sup> | C <sup>+</sup> E <sup>-</sup> | C <sup>-</sup> E <sup>+</sup> | C <sup>-</sup> E <sup>-</sup> | Total colonies | C <sup>+</sup> | E <sup>+</sup> |
|------------------------------------------|------------------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------|----------------|----------------|
|                                          |                                          |                              | C <sup>R</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>S</sup> E <sup>R</sup> |                |                |                |
| IL187-7A                                 | 55R5-3C/221                              | 0                            | 80.2                          | 16.2                          | 0.4                           | 3.2                           | 253            | 96.4           | 80.6           |
|                                          |                                          | 320                          | 63.6                          | 32.4                          | 0.0                           | 4.0                           | 327            | 96.0           | 63.6           |
| $\alpha$                                 | a                                        | 640                          | 56.9                          | 37.0                          | 0.0                           | 6.1                           | 297            | 93.9           | 56.9           |
| $\omega^+$ C <sup>R</sup> E <sup>S</sup> | $\omega^-$ C <sup>S</sup> E <sup>R</sup> | 960                          | 45.1                          | 42.7                          | 0.0                           | 12.2                          | 213            | 87.8           | 45.1           |
| Ir. 5                                    |                                          | 1600                         | 35.0                          | 46.1                          | 0.9                           | 18.0                          | 217            | 81.1           | 35.9           |
|                                          |                                          |                              | C <sup>S</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>R</sup> E <sup>S</sup> |                |                |                |
| DP1-1B/553                               | 55R5-3C/321                              | 0                            | 92.9                          | 4.5                           | 0.0                           | 2.7                           | 224            | 97.3           | 92.9           |
|                                          |                                          | 320                          | 81.1                          | 11.3                          | 0.0                           | 7.7                           | 222            | 92.3           | 81.1           |
| $\alpha$                                 | a                                        | 640                          | 61.6                          | 19.0                          | 0.0                           | 19.4                          | 216            | 80.6           | 61.6           |
| $\omega^+$ C <sup>S</sup> E <sup>R</sup> | $\omega^-$ C <sup>R</sup> E <sup>S</sup> | 960                          | 59.0                          | 10.1                          | 0.0                           | 30.9                          | 188            | 69.1           | 59.0           |
| Ir. 15                                   |                                          | 1600                         | 49.5                          | 11.4                          | 0.0                           | 39.1                          | 289            | 60.9           | 49.5           |

Quantitative random analysis by replica plating onto antibiotic containing media (N3, N3C, N3E, N3CE) of different two point heterosexual crosses including the C<sub>36</sub><sup>R</sup>, C<sub>321</sub><sup>R</sup>, E<sub>221</sub><sup>R</sup>, E<sub>514</sub><sup>R</sup> and E<sub>553</sub><sup>R</sup> mutations. Each row gives, for each dose of UV applied to one of the parents prior to mating, the frequency of the different classes amongst the diploids issued from the cross (in %), the total number of colonies scored and the frequency of the mitochondrial alleles originated from the  $\omega^+$  parent (in %).

Table 5a: the  $\omega^+$  parent irradiated prior to mating;

Table 5b: the  $\omega^-$  parent irradiated prior to mating.

Irradiation numbers (Ir.) refer to Tables 2a and 2b.

class between C and E markers as already discussed for two point crosses i.e. the frequency of this class of recombinants (C<sup>+</sup>E<sup>-</sup>) increases and reaches a maximum value around 50%. The maximum value of recombinants between C and E is therefore less than the maximum value of recombinants between C and O. UV effects on the minor recombinant classes (C<sup>-</sup>E<sup>+</sup>) and (C<sup>-</sup>O<sup>+</sup>) can not be studied easily since their frequency is very low and the relative sampling error is very high. However it can be concluded that the polarity of recombination between C,O and C,E markers is high in all cases and that UV does not introduce major changes in polarity. For the E-O pair of markers effects of UV on both classes of recombinants can be studied. When increasing doses of UV are applied to the  $\omega^+$  parent the frequency of E<sup>+</sup>O<sup>-</sup> recombinant slightly increases up to a maximum and then decreases. Although the relative sampling errors are greater the same phenomenon appears for the E<sup>-</sup>O<sup>+</sup> recombinant class. The maximum frequency of recombinants between E and O is thus observed for low UV doses while after high UV doses the frequency of E O recombinants is smaller than in the controls. Slight variations of polarity of recombination are seen but no major changes are observed.

In conclusion: UV irradiation shows striking differences between homosexual and heterosexual crosses. In homosexual crosses UV equally affects the transmission of all mitochondrial alleles: there is a *coordinated transmission*. In heterosexual crosses effects of UV on transmission of mitochondrial alleles

Table 5 b

| Irradiated parent                        | Non irradiated parent                    | UV dose erg mm <sup>-2</sup> | C <sup>+</sup> E <sup>+</sup> | C <sup>+</sup> E <sup>-</sup> | C <sup>-</sup> E <sup>+</sup> | C <sup>-</sup> E <sup>-</sup> | Total colonies | C <sup>+</sup> | E <sup>+</sup> |
|------------------------------------------|------------------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------|----------------|----------------|
|                                          |                                          |                              | C <sup>S</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>R</sup> E <sup>S</sup> |                |                |                |
| 55R5-3C/321                              | DP1-1B/514                               | 0                            | 73.2                          | 24.7                          | 0.5                           | 1.5                           | 194            | 97.9           | 73.7           |
| a                                        | $\alpha$                                 | 320                          | 83.0                          | 16.0                          | 0.9                           | 0                             | 212            | 99.0           | 83.9           |
| $\omega^-$ C <sup>R</sup> E <sup>S</sup> | $\omega^+$ C <sup>S</sup> E <sup>R</sup> | 640                          | 99.0                          | 1.0                           | 0                             | 0                             | 296            | 100.0          | 99.0           |
| Ir. 26                                   |                                          |                              |                               |                               |                               |                               |                |                |                |
| 55R5-3C/321                              | DP1-1B/514                               | 0                            | 76.7                          | 19.4                          | 0.4                           | 3.5                           | 283            | 96.1           | 77.1           |
|                                          |                                          | 640                          | 81.3                          | 18.0                          | 0                             | 0.7                           | 284            | 99.3           | 81.3           |
| a                                        | $\alpha$                                 | 960                          | 85.0                          | 14.0                          | 0                             | 0.9                           | 214            | 99.0           | 85.0           |
| $\omega^-$ C <sup>R</sup> E <sup>S</sup> | $\omega^+$ C <sup>S</sup> E <sup>R</sup> | 1600                         | 86.3                          | 12.4                          | 0                             | 1.4                           | 291            | 98.7           | 86.3           |
| Ir. 27                                   |                                          |                              |                               |                               |                               |                               |                |                |                |
| 55R5-3C/321                              | IL17-1D                                  | 0                            | 85.2                          | 14.8                          | 0                             | 0                             | 209            | 100.0          | 85.2           |
|                                          |                                          | 320                          | 88.8                          | 11.2                          | 0                             | 0                             | 178            | 100.0          | 88.8           |
| a                                        | $\alpha$                                 | 640                          | 92.1                          | 6.9                           | 0                             | 1.0                           | 203            | 99.0           | 92.1           |
| $\omega^-$ C <sup>R</sup> E <sup>S</sup> | $\omega^+$ C <sup>S</sup> E <sup>R</sup> | 960                          | 90.2                          | 9.8                           | 0                             | 0                             | 215            | 100.0          | 90.2           |
| Ir. 28                                   |                                          | 1600                         | 93.8                          | 6.2                           | 0                             | 0                             | 178            | 100.0          | 93.8           |
| IL16-10B                                 | IL17-9B                                  | 0                            | 67.9                          | 29.0                          | 0.4                           | 2.7                           | 224            | 96.9           | 68.3           |
|                                          |                                          | 320                          | 66.1                          | 28.3                          | 0.6                           | 5.0                           | 180            | 94.4           | 66.7           |
| $\alpha$                                 | a                                        | 640                          | 63.1                          | 29.5                          | 0.5                           | 6.9                           | 217            | 92.6           | 63.6           |
| $\omega^-$ C <sup>R</sup> E <sup>S</sup> | $\omega^+$ C <sup>S</sup> E <sup>R</sup> | 960                          | 76.2                          | 21.6                          | 0                             | 2.2                           | 227            | 97.8           | 76.2           |
| Ir. 30                                   |                                          | 1600                         | 68.9                          | 27.5                          | 1.2                           | 2.4                           | 167            | 96.4           | 70.1           |
| IL16-11D                                 | DP1-1B/514                               | 0                            | 76.6                          | 22.3                          | 0                             | 1.1                           | 269            | 98.9           | 76.6           |
|                                          |                                          | 320                          | 85.9                          | 13.4                          | 0                             | 0.7                           | 276            | 99.3           | 85.9           |
| a                                        | $\alpha$                                 | 640                          | 82.1                          | 16.1                          | 0.4                           | 1.4                           | 285            | 98.2           | 82.5           |
| $\omega^-$ C <sup>R</sup> E <sup>S</sup> | $\omega^+$ C <sup>S</sup> E <sup>R</sup> | 960                          | 85.5                          | 12.9                          | 0                             | 1.6                           | 186            | 98.4           | 85.5           |
| Ir. 31                                   |                                          | 1600                         | 83.3                          | 16.4                          | 0                             | 0.3                           | 299            | 99.7           | 83.3           |
|                                          |                                          |                              | C <sup>R</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>S</sup> E <sup>R</sup> |                |                |                |
| 55R5-3C/221                              | LM650-2C/36                              | 0                            | 94.4                          | 3.7                           | 0                             | 1.9                           | 215            | 98.1           | 94.4           |
|                                          |                                          | 640                          | 95.2                          | 4.3                           | 0                             | 0.5                           | 207            | 99.5           | 95.2           |
| a                                        | $\alpha$                                 | 960                          | 93.5                          | 2.8                           | 0                             | 3.7                           | 217            | 96.3           | 93.5           |
| $\omega^-$ C <sup>S</sup> E <sup>R</sup> | $\omega^+$ C <sup>R</sup> E <sup>S</sup> | 1600                         | 96.5                          | 2.0                           | 0                             | 1.6                           | 254            | 98.5           | 96.5           |
| Ir. 32                                   |                                          |                              |                               |                               |                               |                               |                |                |                |

depend on the distance of the marker considered from  $\omega$ : there is *differential decrease of transmission*. The closer a marker is to  $\omega$  the less its transmission is diminished by UV irradiation of the  $\omega^+$  parent. This is depicted in Fig. 3a, b and c.

In homosexual crosses irradiation of one of the parents diminishes the total frequency of recombinants for any pair of markers considered while in heterosexual crosses the effects of irradiation depend both on whether the  $\omega^+$  or the  $\omega^-$  parent is irradiated and on the pair of markers considered.

### *III. Effects of UV Irradiation of Zygotes just after Mating on the Outcome of the Cross*

Heterosexual two-point (CE) and three-point (CEO) crosses have been performed in which populations of zygotes have been submitted to UV irradiations just after their formation. Synchronous zygotes were prepared as described in Materials and Methods. Zygote populations were harvested and irradiated some 2 to 4 hours after the time of mixing of the two parents. Under these conditions the great majority of zygotes were younger than 1 hour. Further copulations of haploid cells were prevented by diluting and plating the irradiated suspensions.

Table 7a shows the results of three experiments of irradiations of zygotes of the cross DP1-1B/514  $\times$  55R5-3C/321. No significant effects of UV are observed either on the transmission of the C<sup>+</sup> and E<sup>+</sup> alleles or on the frequency of recombinants. The polarity of recombination is unchanged.

Table 7b shows the results of irradiation of zygotes of the cross IL779-1C  $\times$  IL16-10B. For all pairs of markers considered no significant effect of UV on the frequency of recombinants is observed. A minor effect on allele transmission exists (specially for the O<sup>+</sup> allele).

In conclusion UV applied to zygotes after their formation has no effect or only minor effects on the outcome of the cross.

## **Discussion**

The aim of this work was twofold: first to describe the effects of UV irradiation of one of the parents on the phenomena of transmission and recombination of mitochondrial genes, second to discuss these effects in terms of a general model for segregation and recombination of mitochondrial genes. Hypotheses which take into account the molecular basis of the UV action give some insight into the mechanism of recombination of mitochondrial genes and will be discussed in the last section.

### *I. Major Effects of UV Irradiation of One Parent Prior to Mating*

#### **1. Frequency of UV Induced Petites**

In agreement with previous findings (see for ex. Raut and Simpson, 1955) results reported here show that petites are induced by UV irradiation with a high efficiency. Our results also show that for the same UV dose the frequency of petites induced varies markedly between non isogenic strains. Deutsch *et al.* (1974) have reported detailed studies on the kinetics of  $\rho^-$  induction and on the loss of mitochondrial markers after treatment with ethidium bromide. The same type of kinetic analysis based on a multiple target theory (Atwood and Norman, 1949) applies here for the kinetics of  $\rho^-$  induction after UV mutagenesis in various strains (in total 22 different strains have been studied). The data presented in Table 2b permit to reach the same conclusions as Deutsch *et al.* (1974), namely that the mutagenic event has a large size and that the number of targets calculated per cell is small (from 0.9 to 9.4 according to the strains and in average 3.3).

#### **2. Effects of UV on the Output of Markers**

In all experiments the transmission of alleles (*output*) originated from the irradiated parent diminishes as a function of UV dose (see Fig. 2 and Tables 3a, b;

Table 6. Effects of UV on heterosexual three-point crosses

| Irradiated parent                                       | Non irradiated parent | UV dose erg mm <sup>-2</sup> | C+E+O+ | C+E+O- | C+E-O+ | C-E-O+ | C-E-O- | Total colonies | C+   | E+   | O+   |
|---------------------------------------------------------|-----------------------|------------------------------|--------|--------|--------|--------|--------|----------------|------|------|------|
|                                                         |                       |                              | CSESOR | CSESOS | CSESOR | CSESOR | CSESOS |                |      |      |      |
| DP1-1B/7                                                | IL126-1C              | 0                            | 71.6   | 21.3   | 0.0    | 7.1    | 0.0    | 169            | 100  | 92.9 | 71.6 |
|                                                         |                       | 320                          | 58.6   | 25.3   | 2.0    | 14.1   | 0.0    | 198            | 100  | 83.8 | 60.6 |
|                                                         | $\alpha$              | 640                          | 35.8   | 30.6   | 4.2    | 25.7   | 0.4    | 265            | 96.2 | 67.2 | 40.8 |
|                                                         | $\omega^+$ CSESOR     | 960                          | 18.8   | 38.3   | 2.6    | 29.2   | 0.0    | 154            | 89.0 | 58.4 | 21.4 |
| Ir. 16                                                  |                       | 1600                         | 4.8    | 25.2   | 0.0    | 51.0   | 0.0    | 147            | 81.0 | 30.6 | 4.8  |
| DP1-1B/7                                                | IL126-1C              | 0                            | 54.5   | 30.7   | 1.1    | 12.7   | 0.0    | 189            | 98.9 | 85.2 | 55.6 |
|                                                         |                       | 320                          | 35.5   | 24.5   | 1.5    | 34.0   | 0.0    | 200            | 95.5 | 60.5 | 37.0 |
|                                                         | $\alpha$              | 640                          | 21.4   | 29.1   | 2.0    | 39.8   | 0.5    | 196            | 92.3 | 51.5 | 24.0 |
|                                                         | $\omega^+$ CSESOR     | 960                          | 14.2   | 19.8   | 0.5    | 50.3   | 0.0    | 197            | 84.8 | 34.5 | 15.2 |
| Ir. 17                                                  |                       | 1600                         | 4.9    | 11.0   | 1.2    | 36.6   | 0.0    | 164            | 53.7 | 16.5 | 6.1  |
| IL836-2A                                                | IL126-3A              | 0                            | 48.4   | 37.0   | 2.1    | 12.0   | 0.0    | 192            | 99.5 | 85.9 | 50.5 |
|                                                         |                       | 320                          | 27.1   | 41.2   | 2.8    | 23.2   | 0.6    | 177            | 94.4 | 70.1 | 30.5 |
|                                                         | $\alpha$              | 640                          | 19.2   | 47.7   | 1.7    | 28.8   | 0.3    | 344            | 97.4 | 67.4 | 21.2 |
|                                                         | $\omega^+$ CSESOR     | 960                          | 14.8   | 45.4   | 5.2    | 29.1   | 0.2    | 405            | 94.6 | 62.0 | 20.7 |
| Ir. 21 <sup>a</sup>                                     |                       | 1600                         | 11.1   | 48.0   | 4.1    | 32.4   | 0.4    | 244            | 95.5 | 60.7 | 15.6 |
| CSESOR CSESOS CSESOR CSESOS CSESOR CSESOS CSESOR CSESOS |                       |                              |        |        |        |        |        |                |      |      |      |
| IL779-1C                                                | IL16-10B              | 0                            | 28.0   | 24.5   | 9.1    | 34.3   | 0.0    | 143            | 95.8 | 53.8 | 37.1 |
|                                                         |                       | 320                          | 13.3   | 27.8   | 5.0    | 49.4   | 0.0    | 180            | 95.6 | 41.1 | 18.3 |
|                                                         | $\alpha$              | 640                          | 13.7   | 19.6   | 8.3    | 50.0   | 0.0    | 204            | 91.7 | 33.3 | 22.1 |
|                                                         | $\omega^+$ CSESOR     | 960                          | 9.8    | 15.7   | 5.9    | 58.8   | 0.0    | 153            | 90.2 | 26.1 | 15.7 |
| Ir. 23                                                  |                       | 1600                         | 7.8    | 15.7   | 10.8   | 46.1   | 0.0    | 102            | 80.4 | 23.5 | 20.6 |

| C <sup>SE</sup> S <sup>OR</sup> C <sup>SE</sup> S <sup>OS</sup> C <sup>SE</sup> R <sup>OR</sup> C <sup>SE</sup> R <sup>OS</sup> C <sup>R</sup> E <sup>SE</sup> R <sup>OR</sup> C <sup>R</sup> E <sup>SE</sup> R <sup>OS</sup> C <sup>R</sup> E <sup>R</sup> O <sup>R</sup> C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> |                                                         |      |      |      |      |      |     |     |     |      |     |      |      |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|------|------|------|------|------|-----|-----|-----|------|-----|------|------|------|
| D22/A16                                                                                                                                                                                                                                                                                                                 | IL126-3A                                                | 0    | 64.2 | 20.5 | 4.8  | 10.5 | 0.0 | 0.0 | 0.0 | 0.0  | 229 | 100  | 84.7 | 69.0 |
|                                                                                                                                                                                                                                                                                                                         |                                                         | 320  | 18.8 | 30.5 | 11.3 | 38.5 | 0.0 | 0.0 | 0.0 | 0.5  | 213 | 99.1 | 49.8 | 30.0 |
| a                                                                                                                                                                                                                                                                                                                       | $\alpha$                                                | 640  | 8.5  | 18.9 | 5.6  | 59.7 | 0.0 | 0.0 | 0.8 | 6.5  | 355 | 92.7 | 27.3 | 14.9 |
| $\omega^+$ C <sup>SE</sup> S <sup>OR</sup>                                                                                                                                                                                                                                                                              | $\omega^-$ C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | 960  | 6.7  | 12.3 | 2.2  | 69.1 | 0.0 | 0.0 | 0.0 | 9.7  | 269 | 90.3 | 19.0 | 8.9  |
| Ir. 20 <sup>a</sup>                                                                                                                                                                                                                                                                                                     |                                                         |      |      |      |      |      |     |     |     |      |     |      |      |      |
| D22/A15                                                                                                                                                                                                                                                                                                                 | IL126-3A                                                | 0    | 51.2 | 29.8 | 3.2  | 15.3 | 0.0 | 0.0 | 0.0 | 0.4  | 248 | 99.6 | 81.0 | 54.4 |
|                                                                                                                                                                                                                                                                                                                         |                                                         | 320  | 19.4 | 33.1 | 6.3  | 38.9 | 0.0 | 0.0 | 0.6 | 1.7  | 175 | 97.7 | 52.6 | 26.3 |
| a                                                                                                                                                                                                                                                                                                                       | $\alpha$                                                | 640  | 5.3  | 30.1 | 3.7  | 54.9 | 0.0 | 0.0 | 0.0 | 6.1  | 246 | 93.9 | 35.4 | 8.9  |
| $\omega^+$ C <sup>SE</sup> S <sup>OR</sup>                                                                                                                                                                                                                                                                              | $\omega^-$ C <sup>R</sup> E <sup>R</sup> O <sup>S</sup> | 960  | 1.3  | 15.9 | 2.8  | 67.2 | 0.0 | 0.0 | 0.3 | 12.5 | 320 | 87.2 | 17.5 | 4.1  |
| Ir. 19 <sup>a</sup>                                                                                                                                                                                                                                                                                                     |                                                         | 1600 | 4.7  | 8.1  | 1.7  | 74.1 | 0.0 | 0.0 | 0.0 | 11.1 | 405 | 88.6 | 13.1 | 6.4  |
| IL126-1C                                                                                                                                                                                                                                                                                                                | DP1-1B/7                                                | 0    | 68.3 | 22.2 | 0.9  | 8.1  | 0.0 | 0.0 | 0.0 | 0.5  | 221 | 99.5 | 90.5 | 69.2 |
|                                                                                                                                                                                                                                                                                                                         |                                                         | 320  | 61.4 | 22.3 | 2.5  | 13.7 | 0.0 | 0.0 | 0.0 | 0.0  | 197 | 100  | 83.8 | 64.0 |
| a                                                                                                                                                                                                                                                                                                                       | $\alpha$                                                | 640  | 59.2 | 27.9 | 2.2  | 10.1 | 0.0 | 0.0 | 0.0 | 0.6  | 179 | 99.4 | 87.2 | 61.5 |
| $\omega^-$ C <sup>R</sup> E <sup>R</sup> O <sup>S</sup>                                                                                                                                                                                                                                                                 | $\omega^+$ C <sup>SE</sup> S <sup>OR</sup>              | 960  | 59.4 | 24.9 | 1.8  | 13.8 | 0.0 | 0.0 | 0.0 | 0.0  | 217 | 100  | 84.3 | 61.3 |
| Ir. 36                                                                                                                                                                                                                                                                                                                  |                                                         | 1600 | 56.6 | 25.9 | 3.5  | 11.9 | 0.0 | 0.0 | 1.4 | 0.7  | 143 | 97.9 | 82.5 | 61.5 |

<sup>a</sup> Crosses performed with collaboration of P. R. Avner (see Avner *et al.*, 1973).

Quantitative random analysis by replica plating onto antibiotic containing media (N3, N3C, N3E, N3O, N3CE, N3CO) of different three point heterosexual crosses including the C<sub>221</sub>, E<sub>221</sub>, O<sub>1</sub>, O<sub>7</sub>, O<sub>14</sub> and O<sub>145</sub> mutations. Each row gives, for each dose of UV applied to one of the parents, prior to mating, the frequency of the different classes amongst the diploids issued from the cross (in %), the total number of colonies scored and the frequency of the mitochondrial alleles originated from the  $\omega^+$  parent (in %).

Irradiation numbers (Ir.) refer to Tables 2a and b.

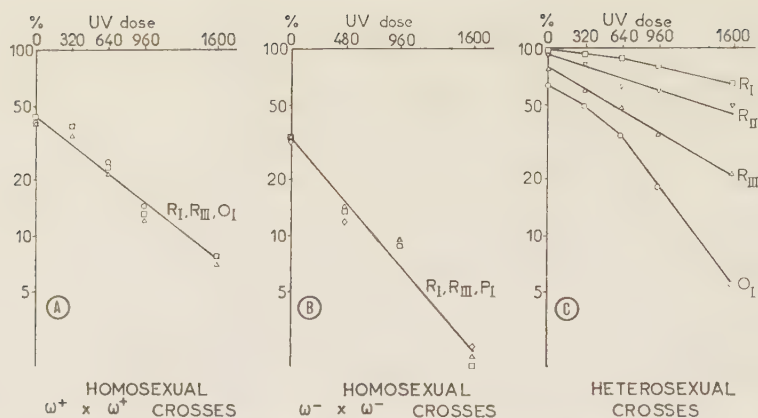


Fig. 3A-C. Comparison of the effects of UV on transmission of alleles of different mitochondrial loci in homosexual and heterosexual crosses. Abscissae: UV dose in  $\text{erg mm}^{-2}$ . Ordinates: transmission of alleles originated from the irradiated parent (in %) plotted on a logarithmic scale

(A) Homosexual cross  $\omega^+ \times \omega^+$ .  $\square$  Transmission of  $C_{321}$  marker ( $R_I$  locus) (average of 3 experiments);  $\Delta$  Transmission of  $E_{514}$  marker ( $R_{III}$  locus) (average of 3 experiments);  $\circ$  Transmission of  $O_7$  marker ( $O_I$  locus) (average of 3 experiments) in the cross DP1-1B/7 irradiated.  $\times$  IL8-8D (Data taken from Table 4)

(B) Homosexual cross  $\omega^- \times \omega^-$ .  $\square$  Transmission of  $C_{321}$  marker ( $R_I$  locus);  $\Delta$  Transmission of  $E_{221}$  marker ( $R_{III}$  locus);  $\diamond$  Transmission of  $P_{454}$  marker ( $P_I$  locus) in the cross M12/54 irradiated.  $\times$  PS 189-3A (Data taken from Table 4)

(C) Heterosexual crosses. In this series of crosses (Data taken from Tables 5 and 6) the irradiated strain was always DP1-1B carrying different mitochondrial mutations.  $\square$  Transmission of  $C_{321}$  marker ( $R_I$  locus) (average of 6 experiments) in the crosses: DP1-1B/514 irradiated.  $\times$  55R5-3C/321, DP1-1B/553 irradiated.  $\times$  55R5-3C/321, DP1-1B/7 irradiated.  $\times$  IL126-1C.  $\nabla$  Transmission of  $E_{553}$  marker ( $R_{II}$  locus) in the cross DP1-1B/553 irradiated.  $\times$  55R5-3C/321.  $\Delta$  Transmission of  $E_{514}$  and  $E_{221}$  markers ( $R_{III}$  locus) (average of 5 experiments) in the crosses: DP1-1B/514 irradiated.  $\times$  55R5-3C/321, DP1-1B/7 irradiated.  $\times$  IL126-1C.  $\circ$  Transmission of  $O_7$  marker ( $O_I$  locus) (average of 2 experiments) in the cross: DP1-1B/7  $\times$  IL126-1C

4; 5a, b; 6). It must be stressed that the decrease of output of alleles at a given locus is independent of whether the irradiated allele is resistant or sensitive. Therefore the decrease does not depend on the nature of the mutation itself.

Furthermore, the decrease depends neither on the function specified by the genetic locus: it concerns both the loci controlling ATPase synthesis (Avner and Griffiths, 1973a and b; Avner *et al.*, 1973; Somlo *et al.*, 1974) and the loci specifying ribosomal function (Grivell *et al.*, 1973; Netter *et al.*, 1974), nor on the location: it concerns as well loci unlinked by genetic recombination (e.g.  $P_I$  and  $O_I$ ) as those linked by genetic recombination (e.g.  $R_I$  and  $R_{III}$ ). The decrease does not depend on whether the irradiated mitochondrial alleles originate from  $\alpha$  or from  $\alpha$  cellular mating type.

However, this decrease may vary greatly from one cross to another when different strains are irradiated with the same UV dose. Each strain has therefore its own degree of sensitivity concerning the decrease of the output of alleles in the cross. This degree of sensitivity is not correlated with the survival of the

irradiated haploid and is not directly correlated with the percent of petites cells induced in the irradiated haploid population.

The decrease of the output is very much dependant on the nature of the cross i.e. whether homosexual ( $\omega^+ \times \omega^+$  or  $\omega^- \times \omega^-$ ) or heterosexual ( $\omega^+ \times \omega^-$ ) and in the later case it is dependant on the position of the locus relatively to  $\omega$ . In all homosexual crosses, both 2-point and 3-point (either  $\omega^+ \times \omega^+$  or  $\omega^- \times \omega^-$ ) the decrease of output of all the markers considered is always coordinated. In the case of heterosexual crosses distinction has to be made between irradiation of the  $\omega^+$  parent and irradiation of the  $\omega^-$  parent. When the  $\omega^+$  parent is irradiated the decrease of  $O$  is always greater than that of  $E$ , itself greater than that of  $C$  (Fig. 3c shows, in an isonuclear genetic background that there is a correlation between the decrease of output and distance of the markers relative to  $\omega$ ). The closer the location of a marker to  $\omega$  the smaller the effect of UV on its output. In an extreme case there is practically no effect on the markers located close to  $\omega$  and there is a maximum effect for markers located far from  $\omega$ . In the case where the  $\omega^-$  parent is irradiated the effects of UV on output are much less pronounced for  $C$  and  $E$  markers in comparison with the effect observed in the irradiation of the  $\omega^+$  parent. However, it should be pointed out that the output of markers  $C$  and  $E$  from the  $\omega^-$  parent is very low even in control crosses. In such cases the decrease in output is more difficult to measure. Only one 3 point cross including a marker more distant from  $\omega$  has been performed. But in this cross the irradiated strain was very insensitive to UV as far as the decrease in output is concerned.

In conclusion the effect of UV on mitochondrial crosses enhances the difference between homosexual and heterosexual crosses and underlines the existence of two different alleles at the  $\omega$  locus.

### 3. Effects of UV on the Frequency of Recombinants

UV irradiation of one parent prior to mating has a significant effect on the frequency of recombinants among diploid progeny of the cross in both homosexual and heterosexual types of crosses. This effect is different depending on the nature of the cross. In all homosexual crosses, both  $\omega^+ \times \omega^+$  and  $\omega^- \times \omega^-$ , the frequency of recombinants decreases as a function of UV dose. The decrease is symmetrical i.e. it exists when either one or the other parent is irradiated. In heterosexual crosses when the  $\omega^+$  parent is irradiated the frequency of recombinants increases while it decreases when the  $\omega^-$  parent is irradiated. In summary, as long as at least one of the genetic markers of the polar region is involved in the cross, the same UV irradiation on an  $\omega^+$  strain can result, for the same pair of loci, in two opposite effects depending on whether the irradiated strain is crossed with an  $\omega^+$  partner or with an  $\omega^-$  one. In the first case the frequency of recombinants diminishes while it increases in the second one. Therefore the drastic modifications of the frequency of recombinants cannot be due to a direct effect of UV on the mechanism of recombination itself (frequency of exchanges between two paired mit-DNA molecules per unit of length per unit of time) but have to be due to some other parameters. Furthermore, when the  $\omega^+$  parent is irradiated the in-

Tables 7a and b. Effects of UV irradiation of zygotes  
Table 7a

| Irradiated<br>zygotes<br>from cross                      | UV dose<br>erg<br>mm <sup>-2</sup> | Survival<br>of<br>zygotes | C <sup>S</sup> E <sup>R</sup> | C <sup>S</sup> E <sup>S</sup> | C <sup>R</sup> E <sup>R</sup> | C <sup>R</sup> E <sup>S</sup> | Total<br>colonies | C <sup>+</sup> | E <sup>+</sup> |
|----------------------------------------------------------|------------------------------------|---------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------|----------------|----------------|
| DP1-1B/514                                               | 0                                  | 100                       | 84.9                          | 14.7                          | 0.4                           | 0.0                           | 238               | 99.6           | 85.3           |
| $\alpha \omega^+ \text{C}^{\text{S}}\text{E}^{\text{R}}$ | 320                                | 86.4                      | 83.0                          | 17.0                          | 0.0                           | 0.0                           | 206               | 100            | 83.0           |
| X                                                        | 640                                | 90.0                      | 83.8                          | 14.6                          | 0.0                           | 1.5                           | 198               | 98.4           | 83.8           |
| 55R5-3C/321                                              | 960                                | 71.8                      | 79.7                          | 18.7                          | 1.0                           | 0.5                           | 187               | 98.4           | 80.7           |
| $\alpha \omega^- \text{C}^{\text{R}}\text{E}^{\text{S}}$ | 1600                               | 17.8                      | 85.0                          | 13.9                          | 0                             | 1.0                           | 194               | 98.9           | 85.0           |
| Ir. 38                                                   | 2400                               | 8.6                       | 82.9                          | 14.6                          | 0.6                           | 1.8                           | 164               | 97.5           | 83.5           |
| DP1-1B/514                                               | 0                                  | 100                       | 71.4                          | 24.8                          | 1.5                           | 2.2                           | 133               | 96.2           | 72.9           |
| $\alpha \omega^+ \text{C}^{\text{S}}\text{E}^{\text{R}}$ | 960                                | 72.6                      | 75.5                          | 21.9                          | 0.4                           | 2.1                           | 233               | 97.4           | 75.9           |
| X                                                        |                                    |                           |                               |                               |                               |                               |                   |                |                |
| 55R5-3C/321                                              | 0                                  | 100                       | 74.3                          | 24.3                          | 0.5                           | 0.9                           | 214               | 98.6           | 74.8           |
| $\alpha \omega^- \text{C}^{\text{R}}\text{E}^{\text{S}}$ | 960                                | 61.8                      | 79.6                          | 17.7                          | 1.6                           | 1.1                           | 186               | 97.3           | 81.2           |
| Ir. 39a and b                                            |                                    |                           |                               |                               |                               |                               |                   |                |                |

crease depends on the distance of the markers from  $\omega$ . The increase is greater between markers located close to  $\omega$  i.e. when the polarity is high.

No or only minor modifications of the polarity of recombination are observed after UV irradiation in both homosexual and heterosexual crosses. However, in a very few cases of homosexual crosses a bias in the frequency of recombinants can be observed. This bias may be interpreted in terms of  $\varrho^-$  induced (see later on) and should be distinguish from polarity of recombination due to the  $\omega$  locus.

In 3 point homosexual crosses a high positive coincidence has already been reported (Kleese *et al.*, 1972 b; Avner *et al.*, 1973; Wolf *et al.*, 1973). In the results presented here a similar coincidence is observed for control crosses. Moreover this coincidence increases after UV irradiation. This will be discuss with the model of recombination.

## II. Interpretation of UV Effects in Terms of a General Model for Recombination

### 1. Parameters of the Model

A general model for recombination and segregation of mitochondrial genes has recently been proposed by Dujon *et al.* (1974). A part of this model is inspired by the Visconti and Delbrück theory for bacteriophage crosses (Visconti and Delbrück, 1953). The model is supported by a quantitative agreement between the predictions it allows and the results of all crosses performed. This model stresses that mitochondrial genetics are genetics of a population of mit-DNA molecules forming a panmictic pool and undergoing several mating rounds. It stresses also that the variations of output observed from one cross to another can be interpreted as representative of variations of input (proportions of molecules of one parent relative to that of the other at time zero of the cross). In such a case the final frequency of recombinants is both a function of the probabilities of genetic exchanges between two molecules and a function of all parameters which can influence the number of heterozygous pairings within the pool. Furthermore,

Table 7b

| Irradiated<br>zygotes<br>from cross | UV dose<br>erg<br>mm <sup>-2</sup> | Survival<br>of<br>zygotes | C+E+O+ | C+E+O- | C+E-O+ | C+E-O- | C-E-O+ | C-E-O- | C-E-O+ | C-E-O- | Total        | O+   |
|-------------------------------------|------------------------------------|---------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------------|------|
|                                     |                                    |                           | CSEOR  | CSEOR  | CSEOR  | CSEOR  | CSEOR  | CSEOR  | CSEOR  | CSEOR  | col-<br>nies |      |
| IL779-1C                            | 0                                  | 100                       | 30.8   | 16.2   | 10.3   | 40.0   | 0.0    | 0.0    | 0.0    | 2.7    | 185          | 41.1 |
| a $\omega^+$ CSEOR                  | 640                                | 51.1                      | 24.2   | 21.6   | 5.2    | 39.9   | 0.0    | 0.0    | 2.0    | 7.2    | 153          | 31.4 |
| X                                   |                                    |                           |        |        |        |        |        |        |        |        |              |      |
| IL16-10B                            |                                    |                           |        |        |        |        |        |        |        |        |              |      |
| $\alpha \omega^-$ CSEOS             | 960                                | 24.6                      | 18.2   | 23.4   | 5.7    | 42.6   | 0.5    | 0.5    | 0.5    | 8.6    | 209          | 24.9 |
| lr. 40                              |                                    |                           |        |        |        |        |        |        |        |        |              |      |

Quantitative random analysis by replica plating onto antibiotic containing media (N3, N3C, N3E, N3O, N3CE, N3CO according to the markers present in the cross) of heterosexual crosses including the C<sub>321</sub>, E<sub>221</sub>, E<sub>514</sub> and O<sub>1</sub><sup>R</sup> mutations. Each row gives, for each dose of UV applied to the zygotes just after their formation, the survival of zygotes (in %), the frequency of the different classes amongst the diploids issued from the cross (in %), the total number of colonies scored and the frequency of the mitochondrial alleles originated from the  $\omega^+$  parent (in %).

Table 7a: 2 point cross; Table 7b: 3 point cross.

lr. 38, lr. 39a, lr. 39b and lr. 40 refer respectively to irradiations performed 4 h 15, 2 h 20, 2 h 50 and 3 h 30 after the mixing of the two parents.

it is assumed that each elementary act of recombination between two mit-DNA molecules is non reciprocal i.e. produces always one parental molecule concomitantly with one recombinant molecule. The polarity of recombination observed is interpreted as the result of several random in time mating rounds, in which all elementary acts of recombination are non reciprocal.

The final outcome of a cross is determined by three types of parameters: a) The average number of mating rounds before segregation in a genetically pure cell, b) The value of input, c) The probabilities of genetic exchanges for a pair of markers between two already paired mit-DNA molecules.

## 2. Variations of Input as a Major UV Effect

As previously discussed the major UV effects are twofold: a) UV influences the output of markers in the cross, b) UV influences the overall frequency of recombinants. The question arises: are the UV effects interpretable in terms of modifications of one or several of the parameters of the model?

A priori, UV irradiation of one of the parents prior to mating might change either the number of mating rounds, or the input fraction, or the probabilities and the nature of genetic exchanges, or even simultaneously several if not all these parameters. UV might also have some influence on other biological processes not taken into account as parameters of the model, but which might be of importance for the final outcome of the cross.

However, the striking fact that UV can significantly either *increase* or *decrease* the frequency of recombinants has lead us to the conclusion that the primary target of UV is not the frequency of genetic exchanges itself but another phenomenon which determines simultaneously the final frequency of recombinants and the output of the alleles. The simplest interpretation is then to assume that the primary effect of UV is to modify the input fraction and thereby to introduce changes in both the output of different alleles and the frequency of recombinants.

## 3. Output of a Given Allele Relative to Output of the Others

Fig. 4 shows the relationship between output of C, E, O and P genetic markers after UV irradiation as well as in control crosses for homosexual and heterosexual crosses.

The model predicts that in homosexual crosses the output of every allele should be proportional to its input and therefore that the output of alleles at different loci carried by the same mit-DNA molecule and originated from the same parent should be identical. This has already been demonstrated in the case of different crosses not involving UV irradiation and has been designated as the *coordinate output* of alleles (Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974; Dujon *et al.*, 1974). Fig. 4a, b and c show that the rule of the coordinate output holds also true after UV irradiation. This rule is applicable for all UV doses, for all homosexual crosses and is independant of the actual value of output. For convenience all the results given in Fig. 4 have been presented showing the output of mitochondrial alleles introduced to the cross by the  $\alpha$  cellular mating type parent. As shown in the results the irradiation concerns either this parent or the  $\alpha$  cellular mating type parent. The rule of coordinate output of alleles after

UV irradiation holds true whether the output has increased after irradiation in comparison to the controls (UV on the  $\alpha$  parent), or whether it has decreased after irradiation (UV on the  $a$  parent). Linear regressions of the experimental data are in good agreement with the predictions of the model (regression coefficients are respectively 0.988, 0.993 and 0.992 for Fig. 4a, 4b and 4c).

In heterosexual crosses the model predicts that outputs of alleles located in non polar regions (such as O) are proportional to their inputs while this is not the case for alleles located in the polar region (such as C and E). The formula relating the output of C and E to their input is given in the legend of Fig. 4. It follows that, in heterosexual crosses, the output of C is different from the output of E, both being different from the output of O. Fig. 4d, e and f show for all UV doses and all crosses performed that variation of output of one allele is accompanied by variation of output of the other allele according to the prediction of the model. For example, when the output of O varies from 1 to 0.15 the output of C should vary from 1 to 0.92 while the output of E should vary from 1 to 0.38. This explains the finding that when applied to an  $\omega^+$  parent UV decreases the output of  $O^+$  more than it decreases the output of  $E^+$  and even more than it decreases the output of  $C^+$  (see Fig. 3). The scattering of experimental points may be due firstly to the sampling error of colonies scored, secondly to slight variations of the parameters  $m$  and  $s$  (which are assumed not to vary) from one cross to another. Fitting the parameters of the model with the experimental data gives the following values:  $m=4.6$ ;  $s_C=0.99$ ;  $s_E=0.50$ . It must be noted that this value of  $m$ , although similar to that calculated from the complete set of crosses (Dujon *et al.*, 1974) is not exactly equal to it ( $m=3.7$ ). This could be due to the sample of crosses studied here, but also it is not excluded that UV might have some little influence on the parameter  $m$  (and possibly  $s$  too).

#### 4. Frequency of Recombinants as a Function of Output of Alleles

The model predicts that the frequency of recombinants and the output of alleles are all functions of the input and hence permits to calculate the theoretical relationship between frequencies of recombinants and output of every alleles for homosexual as well as heterosexual crosses.

In homosexual crosses the model predicts that the frequency of recombinants should be a parabolic function of the input and, since the output of every allele is proportional to the input, that the frequency of recombinants should be a parabolic function of the output. Fig. 5a, b and c show, for all UV doses and all crosses performed, that in spite of the scatter of experimental data the relationship between the frequency of recombinants and the output of alleles are compatible with the theoretical parabolas drawn. ( $\chi^2$  are respectively 111.0 for 116 degrees of freedom, 99.1 for 58 d.f. and 71.8 for 54 d.f. for Figs. 5a, b and c). Crosses involving the strain M12 are not used for the calculation in Fig. 5a. In agreement with previous finding (Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974) showing the genetic linkage between C and E markers and the absence of genetic linkage between the CO, CP, EO, EP and OP pairs, it can be seen also that the maximum frequency of recombinants are similar for the CO, CP, EO and EP pairs

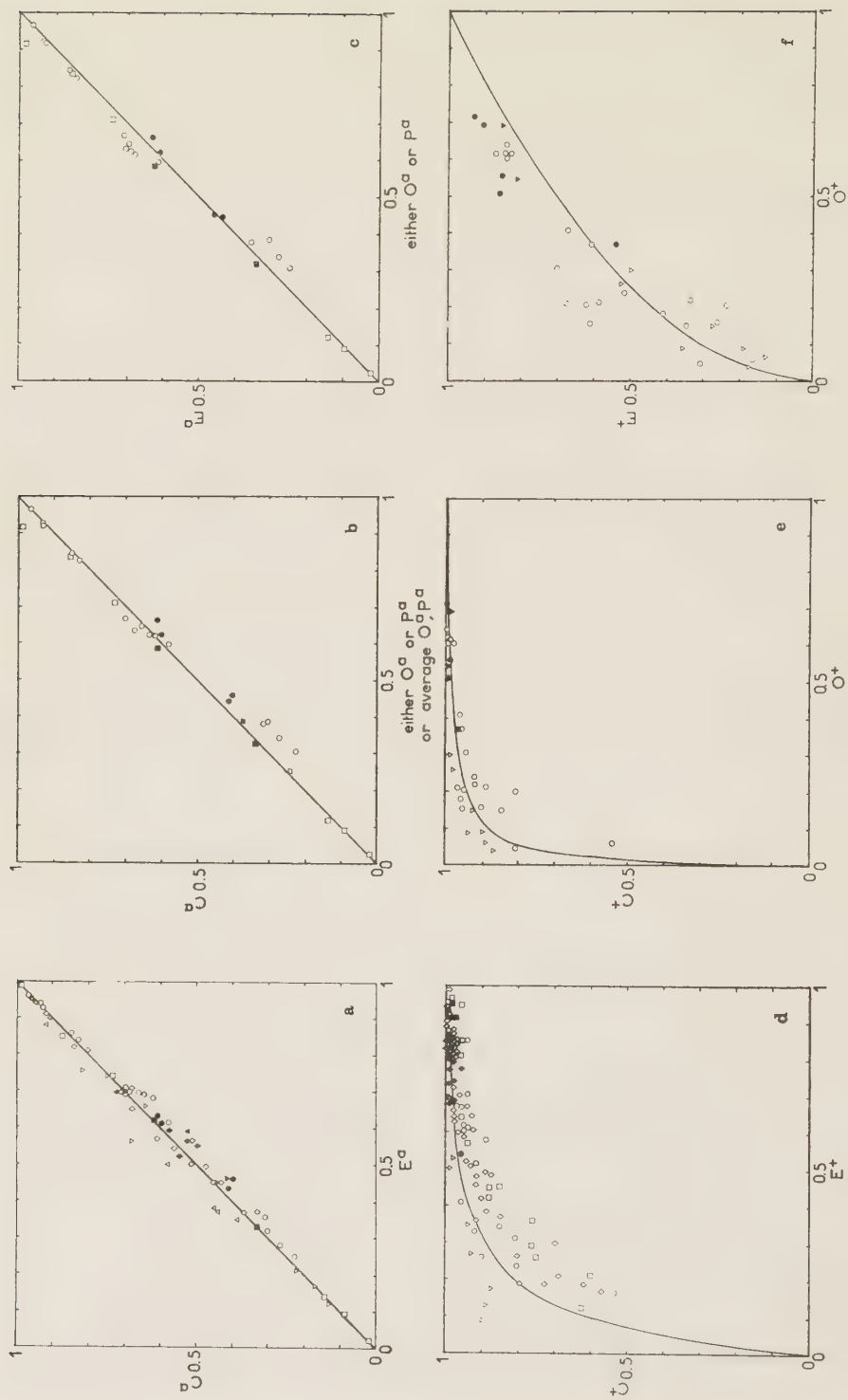


Fig. 4.: a—f

and are greater than the maximum frequency of recombinants for the CE pair. The cross M12/54  $\times$  PS189-3A shows a significantly lower frequency of recombinants between C and E markers than other crosses. The points corresponding to this cross, both UV irradiated and control experiments, do not fit with the parabole drawn on Fig. 5a. This phenomenon has already been reported by Wolf *et al.* (1973) as being a specific property of the M12 strain and appears only for the CE pair and not for the CO and EO pairs.

In heterosexual crosses the model predicts that the relationship between the frequency of recombinants and the input depends on the distance of the genetic markers considered relative to  $\omega$ . When one of the markers, at least, is located in the polar region the maximum frequency of recombinants is observed for an input biased in favor of the  $\omega^-$  parent. Furthermore, the maximum frequency of recombinants is a function of both the distance between the two markers and the distance between  $\omega$  and the marker proximal to it. The model permits also to calculate the relationship between output of alleles and input. Fig. 5d, e and f

Fig. 4. Output fraction of a given allele as a function of the output fraction of other alleles involved in the cross. In all figures the experimental data are compared with the theoretical predictions of the model.

*1 Experimental:* Each point represents the results of a single cross. Full symbols refer to control experiments, void symbols refer to UV irradiations of one of the parents prior to mating.

Abscissae and ordinates: output fraction of the alleles indicated.

Fig. 4a—c: Homosexual crosses (data taken from Tables 3 and 4).  $\diamond$  and  $\blacklozenge$  IL102-8D  $\times$  55R5-3C/321, 55R5-3C/221  $\times$  IL16-10B;  $\nabla$  and  $\blacktriangledown$  IL17-9B  $\times$  IL187-7A;  $\triangle$ ,  $\blacktriangle$  LM650-2C/36  $\times$  IL17-4A;  $\circ$  and  $\bullet$  DP1-1B/7  $\times$  IL8-8D;  $\square$  and  $\blacksquare$  M12/54  $\times$  PS189-3A;  $\diamond$ ,  $\blacklozenge$  KL14-4A  $\times$  IL216-1B.

Fig. 4d—f: Heterosexual crosses (data taken from Tables 5 and 6).  $\diamond$  and  $\blacklozenge$  DP1-1B/514  $\times$  55R5-3C/321, DP1-1B/514  $\times$  IL16-11D, IL17-1D  $\times$  55R5-3C/321, IL17-9B  $\times$  IL16-10B;  $\square$  and  $\blacksquare$  LM650-2C/36  $\times$  55R5-3C/221, IL187-7A  $\times$  55R5-3C/221;  $\bullet$  and  $\circ$  DP1-1B/7  $\times$  IL126-1C, IL836-2A  $\times$  IL126-3A, IL779-1C  $\times$  IL16-10B;  $\nabla$  and  $\blacktriangledown$  D22/A16  $\times$  IL126-3A, D22/A15  $\times$  IL126-3A.

*2 Theoretical.* The lines relate the output fraction (ordinates) of either the C or the E alleles located in a polar region (respectively  $R_I$  and  $R_{III}$  loci) to the output fraction (abscissae) of either the E or the O or the P alleles (respectively  $R_{III}$ ,  $O_I$  or  $O_{II}$  and  $P_I$  loci). They are drawn according to the model which assumes that:

1) The input fraction of all alleles of the same parent carried by the same mit-DNA molecule is the same.

2) In homosexual crosses the output fraction of any allele is equal to its input fraction; therefore the output fractions of all alleles must be equal in any given cross.

3) In heterosexual crosses the output fraction of alleles located in the polar region is related to the input fraction by the formula:

$$R_i = w \left( \frac{e^m}{1 - w + w e^m} \right)^{s_i}$$

where:  $m$  is the average number of mating rounds

$w$  is the input fraction

$R_i$  is the output fraction of a given allele ( $C^+$  or  $E^+$ )

$s_i$  is the probability of gene conversion ( $s_C$  for  $C^- \rightarrow C^+$ ;  $s_E$  for  $E^- \rightarrow E^+$ ).

4) In heterosexual crosses the output fraction of alleles located in non polar regions is equal to their input fraction.

The lines were drawn using the best estimate of the parameters:

$$m = 4.6 \quad s_C = 0.99 \quad s_E = 0.50$$

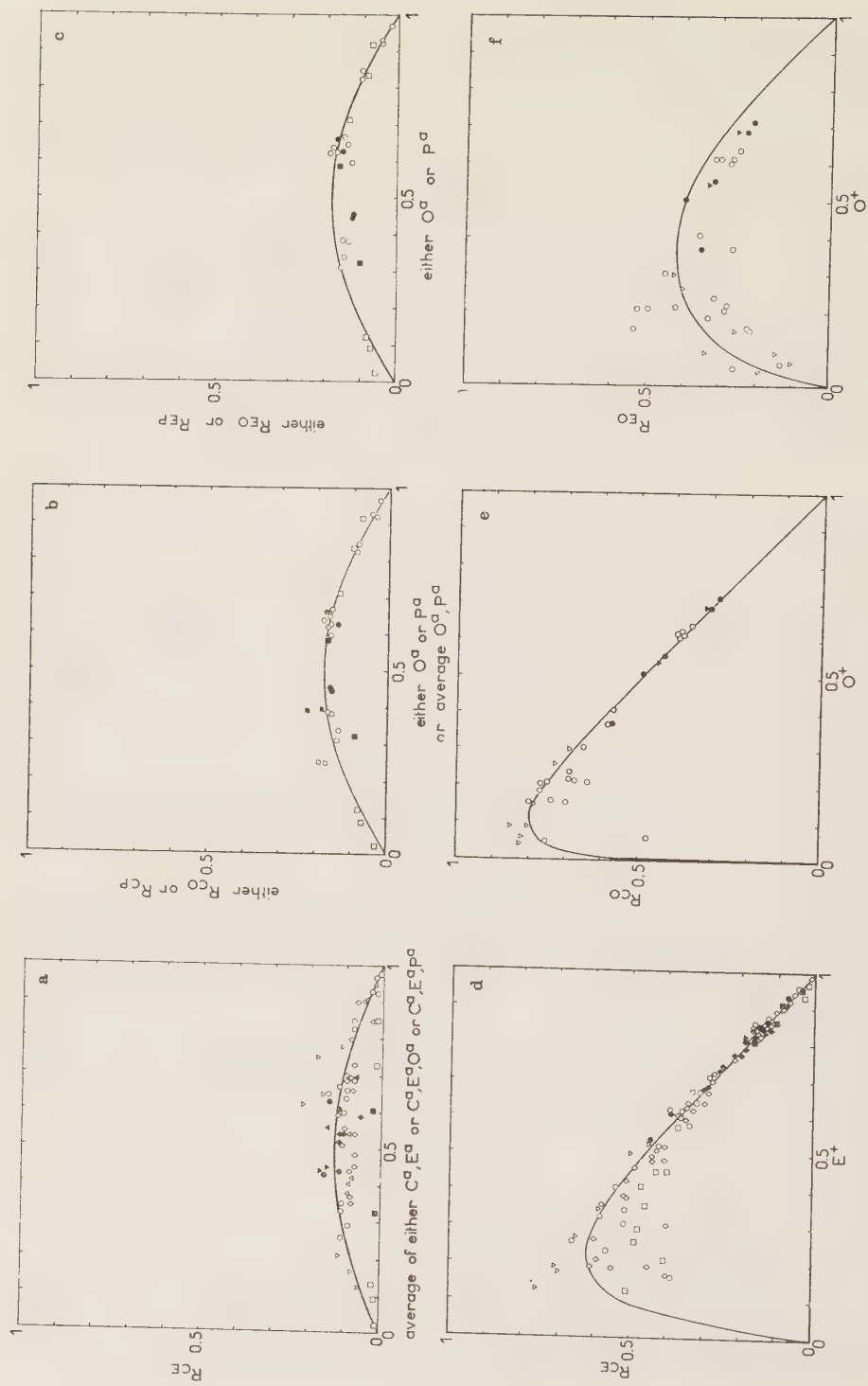


Fig. 5.: a—f

show that, in spite of the scatter of experimental data the relationship between the frequency of recombinants and the output of alleles is compatible with the theoretical equation.

In agreement with crosses performed without UV (Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974) it can be seen also that 1) the maximum frequency of recombinants between the same 2 markers is higher in heterosexual crosses than in homosexual crosses; 2) the maximum frequency of recombinants is higher for the CO pair than for the EO pair i.e. the maximum frequency of recombinants depends on polarity of recombination, 3) the maximum frequency of recombinants between C and E is much lower than between C and O although polarity of recombination is similar indicating the linkage between C and E markers.

### 5. Polarity of Recombination

The model predicts that, whatever the value of the input, no polarity of recombination should be observed in homosexual crosses while in heterosexual crosses, as long as one marker at least of the considered pair, is located in the polar region, the polarity of recombination should exist i.e. there should always be one major recombinant (and always the same one) and one minor recombinant (always the same one). The model predicts also that in the latter case the value of the polarity of recombination between 2 given markers should slightly vary

Fig. 5. Frequencies of recombinant as a function of the output fraction of alleles involved in the cross. In all figures the experimental data are compared with the theoretical predictions of the model.

1 *Experimental*. Fig. 5a, b and c. Homosexual crosses (data taken from Tables 3 and 4) same symbols as in Fig. 4a, b and c.

Fig. 5d, e and f. Heterosexual crosses (data taken from Tables 5 and 6) same symbols as in Fig. 4d, e and f. Ordinates: sum of frequencies of both types of recombinants between the loci indicated. Abscissae: output fraction of the alleles indicated.

2 *Theoretical*. The lines relate the total frequencies of both types of recombinants (ordinates) between either two alleles located in the polar region (CE pair: respectively  $R_I$  and  $R_{III}$  loci) or one allele located in the polar region and one allele located in a non polar region (CO, CP, EO, EP pairs: respectively  $R_I$  and either  $O_I$  or  $O_{II}$ ,  $R_I$  and  $P_I$ ,  $R_{III}$  and either  $O_I$  or  $O_{II}$ ,  $R_{III}$  and  $P_I$  loci) to the output fraction (abscissae) of either the E or the O or the P alleles (respectively  $R_{III}$ ,  $O_I$  or  $O_{II}$  and  $P_I$  loci). They are drawn according to the model which assumes that:

1) In homosexual crosses the frequency of recombinants is related to the input fraction by the formula

$$R_{ij} = 2w(1-w)(1 - e^{-K'_{ij}m})$$

where:  $m$  is the average number of mating rounds;  $w$  is the input fraction;  $R_{ij}$  is the sum of frequencies of both types of recombinants between two alleles (either CE or CO or CP or EO or EP pairs);  $K'_{ij}$  is a factor proportional to the probabilities of genetic exchanges (cf. Dujon *et al.*, in prep.).

2) In heterosexual crosses the frequency of recombinants as a function of the input fraction can be calculated by computer integrations of a complete set of differential equations (cf. Dujon *et al.*, 1974).

3) Output fractions are related to input fractions by assumptions given in legend of Fig. 4.

The lines were drawn using the best estimates of the parameters

$$m: 4.6 \quad s_C: 0.99 \quad s_E: 0.50$$

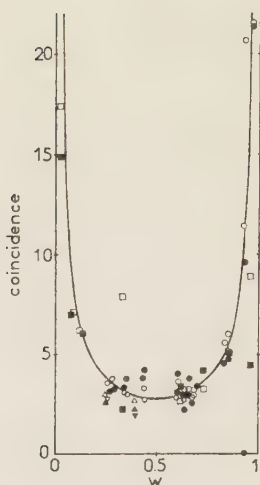


Fig. 6: UV effects on coincidence in three point homosexual crosses. *Experimental*: each point represents the coincidence (i.e. the ratio between the observed double recombinants to the calculated ones) measured in any given cross (control experiments and UV irradiation of one of the parent before mating). DP1-1B/7  $\times$  IL8-8D coincidence calculated assuming respectively CEO and OCE order:  $\circ$ ,  $\bullet$ . M12/54  $\times$  PS189-3A coincidence calculated assuming respectively CEP and PCE order:  $\square$ ,  $\blacksquare$ . KL14-4A  $\times$  IL216-1B coincidence calculated assuming respectively CPO, PCO and CPO order:  $\triangle$ ,  $\blacktriangle$ ,  $\nabla$ . Abscissa: average output of either C, E, O or C, E, P or C, O, P alleles originated from the *a* parent. Ordinate: coincidence. Data taken from Table 4.

*Theoretical*: The curve relates the coincidence to the input fraction. It is drawn according to the model which assumes that: 1°) in homosexual crosses the output fraction of all alleles is equal to their input fraction; 2°) the coincidence is related to the input fraction by the formula

$$CF = \frac{1}{2w(1-w)} \times Q$$

where

$$\left\{ \begin{array}{l} CF \text{ is the coincidence} \\ w \text{ is the input} \\ Q = \frac{(1 - e^{-K'_{ij}K'_{jk}m})}{(1 - e^{-K'_{ij}m})(1 - e^{-K'_{jk}m})} \end{array} \right.$$

Abscissa: input fraction. Ordinate: coincidence. The line was drawn using the best estimate of the parameter  $Q = 1.4$ . Other symbols as in Fig. 5.

as a function of input. In heterosexual crosses the variation of the polarity of recombination as a function of the UV dose is difficult to study since the frequency of the minor recombinant class is very low and consequently the sampling error is relatively very important. However, it is clear that whatever the bias in input introduced by UV irradiation, the polarity is high when the C marker is involved (CE or CO pair) and is smaller when the E marker is involved (EO pair). In homosexual crosses, no significant polarity of recombination is observed after UV irradiation for any pair of markers considered. In conclusion, after UV irradiation, polarity of recombination is compatible with that predicted by the model.

## 6. Positive Coincidence in 3 Point Homosexual Crosses

It is a prediction of the model that the double recombinants observed should be much more frequent than expected from the products of frequencies of the two single recombinants. This phenomenon of high positive coincidence has been demonstrated for all homosexual crosses studied (Kleese *et al.*, 1972b; Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974) and for most of the crosses the coincidence factor found was about 2 to 3. It is also predicted from the model that the value of coincidence is a function of input. As shown on Fig. 6 the coincidence is the lowest for an input of 0.5 but the values are nearly the same for a wide input range (0.3 to 0.7) while it increases very sharply for very biased input (less than 0.05 or more than 0.95). Because no or very few crosses show, in normal conditions, such highly biased input, it was not possible to determine whether experimental coincidence follows the theoretical one. It is interesting therefore to study the coincidence in crosses where a high UV dose has been applied to one parent and thereby where the input is very biased. As predicted by the model, Fig. 6 shows that, although there is some scatter of experimental points (due mainly to the relatively small number of recombinant colonies scored), the coincidence is much higher for crosses with highly biased input than for other crosses.

It can also be seen on Fig. 6 that the coincidence does not differ significantly for CEO, CEP and COP crosses. Nor does it depend on whether the order postulated for calculating coincidence is CEO or OCE, CEP or PCE and COP or CPO or PCO. Thus irradiation does not permit to deduce the order of the O and the P loci relatively to the CE segment.

## 7. Conclusion

In conclusion, UV effects on output of different alleles and on recombinant frequencies between alleles of different loci are in agreement with the theoretical relationships between input, output and recombinant frequency predicted by the model in both homosexual and heterosexual crosses. This is consistent with the idea that the primary effect of UV irradiation is an important modification of the input which is the cause of the modifications of output and modifications of the frequency of recombinants observed. Beside this drastic effect on the input UV irradiation has little or no effects on the frequency of genetic exchanges itself. This idea is confirmed by the fact that irradiation of zygotes after their formation does not significantly modify the frequency of recombinants.

### *III. Interpretation of UV Effects on Input and Petite Induction at the Molecular Level*

Basic ideas of the model are that a) each cell contains a population of many mit-DNA molecules (the average number is around 50 for a haploid cell and around 100 for a diploid cell (Williamson, 1970; Grimes *et al.*, 1974) i.e. there is an inter-molecular redundancy of the mitochondrial genetic information and b) recombination between molecules of mit-DNA occurs all the time i.e. as well in haploid cells as in diploid cells and as well in  $q^+$  cells as in  $q^-$  cells (cf. Michaelis *et al.*, 1973). However, as long as the population of molecules within a cell is homozygous, the molecular recombination is genetically not detectable. Intracellular hetero-

genity leading to the possibility of genetic detection of the molecular recombination events can be introduced in two ways: a) by a cross between two cells, each homogeneous, but carrying different mitochondrial genetic lines; b) by mutagenic treatments affecting different molecules of the same cell. The experiments reported here concern the effects of a combination of these two heterogeneities: a first heterogeneity introduced by the UV treatment and a second heterogeneity introduced by the mating of these already heterogeneous cells with the homogeneous ones carrying different genetic markers.

Intracellular heterogeneity introduced by UV in the population of mit-DNA molecules of irradiated cells results essentially from two steps. The first one, non enzymatic, is the production of pyrimidine dimers in the irradiated DNA molecules as demonstrated by many authors. The second one, metabolic, involves many enzymatic activities which operate on the irradiated DNA molecules (excision, degradation, repair, recombination, etc. ...). These latter steps have been extensively investigated in various organisms, and in the mit-DNA of yeast (for recent review, see Moustacchi *et al.*, 1974).

Both steps could contribute to a decrease in the input of mitochondrial alleles coming from the irradiated parent. The presence of non excised pyrimidine dimers may diminish the "genetic competence" of irradiated molecules i.e. dimers may prevent entering of molecules into the mating pool by several mechanisms such as lethality of the molecules containing dimers, physical compartmentation of the irradiated molecules, slower replication rate of molecules containing dimers, recognition and elimination from the pool of the molecules tagged with dimers, difficulties in pairing and recombination, etc. ... However the enzymatic consequences of the presence of dimers seem more prone to account for variations of input of genetic information. Excised molecules if not repaired rapidly may be degraded and obviously the genetic information they carry must be lost. It is known that the degradation process is relatively rapid after UV irradiation while the repair process may be quite slow (Moustacchi *et al.*, 1974; Waters and Moustacchi, 1974). Molecules which are repaired may often be repaired in an erroneous manner leading to deletions of mit-DNA sequences and to the formation of  $\rho^-$  molecules. The details of the mechanism leading to the formation of  $\rho^-$  cells displaying simultaneous deletions and repetitions of DNA-sequences (Faye *et al.*, 1973) are not known, except that the process is complex and involves several steps (for review see Mahler, 1973). Finally molecular recombination which occurs in every cell may contribute to eliminate the mutated sequences and may lead to a certain kind of reactivation of the irradiated alleles. Non-reciprocal elementary acts of recombination can easily lead to this reactivation.

All these processes, triggered by UV, can lead both to  $\rho^-$  cells when the irradiated population is not mated and to diminution of the quantity of genetic information brought into the cross by the irradiated parent, when the irradiated cell population is mated to a non irradiated parent.

This is depicted in a schematic and oversimplified way in Fig. 8 where deletions of the sequence affecting or not a given genetic marker are shown.

Top line represents haploid cells before mating. Each haploid cell after irradiation contains a heterogeneous population of mit-DNA molecules affected in

different ways. For simplicity of the figure only deletions of sequences have been shown, even though other rearrangements of the sequence are conceivable. Each of the irradiated cells contains a heterogeneous population of molecules and, if grown without mating, gives rise, to a clone (designated as primary clone) containing a heterogeneous population of cells, composed at that time of homogeneous populations of molecules. Recombination and segregation of molecules take place during the growth of the primary clone. Heterogeneity among primary clones issued from mutagenized cells has been reported in the case of ethidium bromide mutagenesis by Nagley *et al.*, 1972 and Deutsch *et al.*, 1974. Although our experience is more limited in the case of UV mutagenized cells, we have found (data not shown) that primary clones are heterogeneous both in respect to  $\rho^+$  vs.  $\rho^-$  cells they contain and in respect to the genetic markers they retain. Furthermore, Deutsch *et al.*, 1974, have found that the essential features of UV mutagenesis are very similar to the ethidium bromide mutagenesis. Various types of these primary clones, as disclosed by the replica cross, are indicated above the irradiated cells. For simplicity of the drawing it is assumed that a) as long as one, at least, intact mit-DNA molecule remains in the cell, this cell gives rise to a clone which contains some  $\rho^+$  cells and will appear as a  $\rho^+$  clone by replica cross, b) as long as one, at least, intact  $E^R$  allele remains in the cell, this cell gives rise to a clone which contains some cells with the  $E^R$  allele and will appear as a  $E^R$  clone by replica cross. These assumptions are probably both correct and incomplete in the sense that a cell in which an allele of a given locus has been destroyed in all the molecules will obviously never regenerate this allele while the maintenance of a significant allele in a few copies only out of many molecules in the cell may not be sufficient to insure the transmission of this allele to the progeny. Recombination mechanism could possibly destroy or "reshuffle" the significant DNA sequence leading to the loss of the genetic marker.

The middle line of the figure represents schematically what happens when a population of irradiated cells containing a heterogeneous population of molecules is crossed to a homogeneous population of cells containing a uniform population of molecules. It is probable that the degradation and repair mechanisms triggered in the haploids are still going on, but in addition to it, pairing and recombination events with the non irradiated molecules begin to occur and continue in the diploid cells lineages. The genetic markers which have been affected by the formation of pyrimidines dimers or have been excised but not repaired or have been degraded by nucleases etc. (vide supra) diminish the input of the genetic information coming from the irradiated parent. In other words, UV irradiation diminishes the intermolecular redundancy of genetic information brought into the cross by the irradiated parent. Several pairing and recombination events occur and on the average every molecule issued some 20 cell generations later has undergone 4.6 mating rounds in its linear ascendance before being segregated out in a cell containing homogeneous population of molecules. This figure of 4.6 does not of course mean that molecular recombination stops after 4.6 rounds of mating but simply means that, on the average, the cell becomes homogeneous for mitochondrial genomes and recombination is no longer genetically detectable.

It is not known whether these 4.6 mating rounds correspond to 4.6 cell divisions (which would mean that on the average one mitochondrial mating round

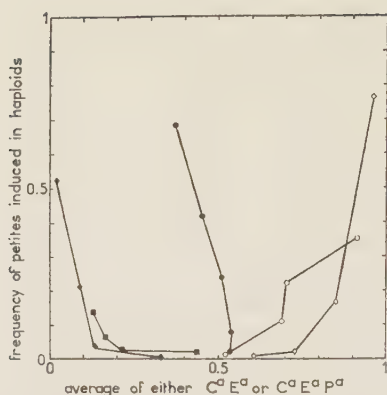


Fig. 7. Examples of relationship observed between the frequency of petites induced in the irradiated haploid parent and the output of mitochondrial alleles in the cross. Each point represents the results of a single cross (control experiments and UV irradiation of one of the parents prior to mating). Abscissa: average output of either C, E or C, E, P mitochondrial alleles originated from the *a* parent. Ordinate: frequency of petites in the irradiated haploid parent. Data taken from Tables 2b, 3 and 4.  $\circ$  IL102-8D irradiated  $\times$  55R5-3C/321;  $\bullet$  55R5-3C/221 irradiated  $\times$  IL16-10B;  $\blacksquare$  IL17-9B irradiated  $\times$  IL187-7A;  $\diamond$  PS189-3A irradiated  $\times$  M12/54;  $\blacklozenge$  M12/54 irradiated  $\times$  PS189-3A

occurs per one cell division) or whether there is more than one mating round per cell division. The other possibility, that there is less than one mating round per cell division is not very probable since it has been shown directly that at the first cell division, a high frequency of pure cells, already occurs (Dujon *et al.*, 1974; Weill *et al.*, in prep.). Therefore, after 4.6 cell generations the great majority of cells are probably pure and recombinational events are no longer genetically detectable.

The last line of the figure represents the situation after the segregations have finished and the different molecular types have been sorted out. At this stage the population of diploid cells is composed almost exclusively of cells containing homogeneous populations of mit-DNA molecules. Thus, the cellular types, scored by replica plating of colonies, correspond to the molecular types of mit-DNA molecule.

In conclusion, the same molecular effects of UV, namely the production of  $\varrho^-$  molecules within irradiated cells, may well explain simultaneously the variation of input in crosses and the production of petites in the vegetative progeny of irradiated cells. This supposes that a simple correlation should be found between the variation of input in crosses and the frequencies of  $\varrho^-$  molecules induced in the irradiated cells. The correlation between variations of input and the frequency of  $\varrho^-$  primary clones (i.e.  $\varrho^-$  cells within irradiated haploid population) may be much more complex since it involves all the processes which, from  $\varrho^-$  molecules, lead to the formation of  $\varrho^-$  cells. However, only this second correlation can be discussed since the frequency of  $\varrho^-$  molecules can not be estimated experimentally while the estimation of  $\varrho^-$  primary clones is very easy.

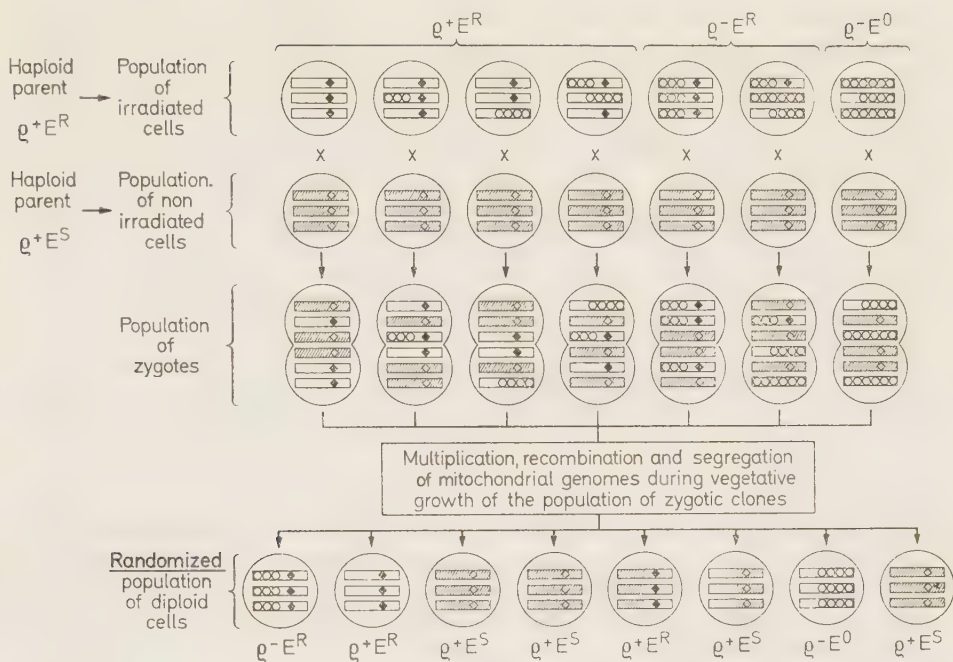


Fig. 8. Schematic representation of the effects of UV applied to one of the parents prior to mating on the evolution of the population of mit-DNA molecules during the cross. Each circle represents one cell, either haploid (top) or diploid (bottom). Each horizontal bar represents one double stranded mit-DNA molecule. Hatched and void bars indicate source of genetic information, not material. Hatched bar: non irradiated parent; void bar: irradiated parent. Lozenges indicate one given mitochondrial locus (the E locus has been taken as example). Full lozenges:  $E^R$  allele; void lozenges:  $E^S$  allele. Parts of the bar filled with circles indicate loss of genetic information corresponding for example to deleted segments of the  $\varrho^-$  molecules. For the sake of simplicity numerous facets of the mitochondrial crosses are not shown by the scheme: packaging of mit-DNA molecules in the organelles; number of mit-DNA molecules which, in a diploid cell, is presumably twice that in a haploid cell; shape of the molecules which are presumably circular; numerous recombination events per molecules and various types of recombined molecules and cells; various patterns of deletions and repetitions in  $\varrho^-$  cells; etc. ...

Fig. 7 shows some examples of the relationship between output of alleles in homosexual crosses (taken as representative of input) and frequency of  $\varrho^-$  primary clones induced in the irradiated parent.

It can be seen that in all cases the greater the frequency of  $\varrho^-$  primary clones induced in the irradiated parent the more biased is the input in favor of the non irradiated one (either  $a$  or  $\alpha$ ). However the form of the relationship observed varies markedly from strain to strain. For IL17-9B or PS189-3A, for example, highly biased input are correlated with high frequency of primary  $\varrho^-$  clones while for 55R5-3C/221 a high frequency of  $\varrho^-$  primary clones is induced in the irradiated population with only minor effect on input. All intermediate cases have been

observed (see Tables 2b, 3a, 3b, 4a and 4b). This considerable variation from strain to strain rules out the trivial possibility that all the effects of UV on output of alleles might be due simply to the existence of  $\rho^-$  cells within the irradiated population (in this case a simple 1 to 1 correlation should be found for every strain) but favors the idea that these effects are due to defective mitochondrial DNA molecules within irradiated cells. The majority of these defective molecules remains in cells which give rise to primary  $\rho^+$  clones. Variations of input from cross to cross according to nuclear genetic backgrounds of the strains (Avner *et al.*, 1973; Wolf *et al.*, 1973; Dujon *et al.*, 1974) might also reflect, in some cases at least, the same kind of phenomenon, i.e. might be due to greater frequencies of  $\rho^-$  molecules within  $\rho^+$  cells in some strains than in others.

It would be of interest to study the relationship between petite frequencies and variations of input in crosses after other treatments than UV (such as ethidium bromide, high glucose repression, inhibitors of mit-DNA synthesis, etc.).

#### *IV. Comparison with Effects of UV on Some Other Genetic Systems*

UV irradiation has been applied as a tool to the study of very different genetic systems such as recombination in bacteriophages, conjugation of bacteria, recombination in eucaryotic chromosomes and extracaryotic inheritance in eucaryotes. Distinction should be made between single-copy-genetic-systems and multiple-copy-genetic-systems. The first refers to cases in which the same genome is represented either once or twice (haploid/diploid), the second refers to cases in which each cell contains many copies of the same genome and in which the number of copies may be variable. The first case is exemplified by the eucaryotic chromosome and the bacterial chromosome while the second is exemplified by bacteriophage crosses and by organelle genetics.

In a single-copy-genetic-system a complete destruction of the unique copy leads to lethality of the cell and such cases are withdrawn from further studies. The action of UV on the mechanism of recombination itself can be studied directly. In a multiple-copy-genetic-system the major effects of UV concern variation of the input and therefore the other effects on the mechanism of recombination itself are not so easily accessible.

Jacob and Wollman (1958) and Joset and Wood (1966) have shown on *E. coli* that when the Hfr parent was irradiated by UV prior to conjugation with an unirradiated  $F^-$  parent, the effects of UV on frequency of the Hfr markers recovered within the recombinant population depend on their distance from the point of initiation for sequential transfer of DNA. The closer a marker is located to the initiation point the less it is affected by UV. Bolotin *et al.* (1971) have reported in the case of two-point heterosexual crosses that when the  $\omega^+$  parent is irradiated prior to mating the output of the C marker is much less influenced by UV than the output of the E marker. This observation has led them to consider a similarity between recombination of mitochondrial genes and bacterial conjugation. However, studies on multifactorial crosses performed later on in this laboratory (Avner *et al.*, 1973; Wolf *et al.*, 1973; Netter *et al.*, 1974) have shown that these similarities do not apply to genetic markers located in other regions of the mitochondrial genome and have made the bacterial conjugation model un-

appealing. Results presented here which concern both the markers of polar and non polar regions confirm that the similarities with bacterial conjugation do not hold true for markers located in non polar regions and are in agreement with a multiple copy model proposed by Dujon *et al.* (1974).

Effects of UV irradiation on bacteriophage genetics [Jacob and Wollman (1955)] show striking similarities with effects of UV irradiation on mitochondrial genetics such as: a) transmission of alleles from the irradiated parent is diminished with increasing doses of UV, b) a certain correlation of the diminution of transmission exist for closely linked markers, c) UV may lead in bacteriophage to complete destruction of large sequences of the genome (about  $10^4$  base pairs long). It is quite possible that a similar mechanism is responsible for petite induction in mitochondria, d) markers may be rescued by recombination events. These similarities strengthen the idea that mitochondrial genetics is the genetics of a population of molecules like bacteriophage genetics.

Finally, it is interesting to compare the results presented here to those described for the non-mendelian genetic system of *Chlamydomonas*. Under normal conditions non-mendelian genes of *Chlamydomonas* show an uniparental mode of inheritance i.e. great majority of zygotes are of the maternal ( $mt^+$ ) with few exceptional biparental zygotes. Sager and Ramanis (1967) have shown that UV treatment of the  $mt^+$  parent prior to mating significantly increases the frequency of biparental zygotes. Following this and other considerations these authors concluded that only 2 copies of extracaryotic genomes enter the zygote (Sager, 1972). More recently Gillham *et al.* (1974), however have shown by a quantitative analysis of their results of UV irradiation that multiple copy models are more prone to explain the experimental data, and have applied to the chloroplast the same type of model as Dujon *et al.* (1974) for mitochondria. Although studies on *Chlamydomonas* are methodologically more comparable to the zygotic clones analysis than to the random analysis of diploid cells which is usually performed in yeast mitochondrial genetics, it is interesting to notice that, in both cases, the results are interpreted on the basis of a population model in which it is supposed that each cell contains multiple and equivalent copies of the organelle genomes.

*Acknowledgements.* The very helpful discussions of P. Avner, W. Lancashire, G. Michaelis, E. Moustacchi, E. Petrochilo and K. Wolf are acknowledged.

One of us (A.K.) gratefully acknowledges the receipt of a fellowship held in Gif as a Boursière de la Convention d'Echanges Centre National de la Recherche Scientifique—Polish Academy of Sciences.

Part of this work has been done when one of us (B.D.) was élève de 4ème année à l'Ecole Normale Supérieure.

This manuscript could not have been completed without the excellent technical assistance of Mrs. S. Chevais and B. Duchaussoy.

This work was supported by a grant ATP Différenciation cellulaire N° 4304 from the C.N.R.S.

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Communicated by W. Gajewski

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